DOES NOT CIRCULATE

olume 59 DECEMBER, 1956 Number 6

UNIVERSITY OF MICHIGAN
DEC 6 1956
HOSPITAL LIBRARY
UNIVERSITY OF MICHIGAN
DEC 6 1956
HOSPITAL LIBRARY

ENDOCRINOLOGY

JÖRGENSEN, C. BARKER, K. G. WINGSTRAND AND PER ROSENKILDE
Neurohypophysis and Water Metabolism in the Toad, *Bufo Bufo* (L.)..... 601
ROMANOFF, ALEXIS L. AND HANS LAUFER
The Effect of Injected Thiourea on the Development of Some Organs of the Chick Embryo..... 611
BATTS, ADRIENNE A., LESLIE L. BENNETT, STANLEY ELLIS AND ROBERT A. GEORGE
Growth Hormone-induced Reduction of Glycosuria and Partial Repair of the Islets of Langerhans in Partially Pancreatectomized Diabetic Rats..... 620
EDGREN, RICHARD A. AND DAVID W. CALHOUN
Density as an Index of the Effects of Estrogens on Bone..... 631
HOUSSAY, B. A. AND J. C. PENHOS
Diabetogenic Action of Pituitary Hormones on Adrenalectomized-Hypophysectomized Dogs..... 637
SCHWEIZER, MALVINA
Effect of Testosterone on Circulating Eosinophils in the Guinea Pig..... 642
SELYE, HANS AND PIERRE BOIS
On the Nephrosclerotic Action of 2-Methyl-9-Fluorocortisol, a Highly Active Artificial Mineralocorticoid..... 646
BERMAN, IRWIN, ABRAHAM EDELMANN AND ALBERT S. GORDON
The Relation of the Adrenal to Phosphorus Metabolism of Bone Marrow... 656
BRODISH, A. AND C. N. H. LONG
Changes in Blood ACTH under Various Experimental Conditions studied by Means of a Cross-circulation Technique..... 666
BIRMINGHAM, MARION K., ERIKA KURLENTS, G. J. ROCHEFORT, M. SAFFRAN AND A. V. SCHALLY
ACTH Content of Rat Pituitary Glands..... 677
GEMZELL, CARL A. AND FRANK HEIJKENSKJÖLD
Growth Hormone Content in Human Pituitaries..... 681
ESKIN, B. A. AND E. M. BOGDANOVE
The Influence of Estrogen upon Goiter Induction in Adult and Immature Rats 688
PINCUS, GREGORY, M. C. CHANG, M. X. ZARROW, E. S. E. HAFEZ AND ANNE MERRILL
Studies of the Biological Activity of Certain 19-nor Steroids in Female Animals 695
NOTES AND COMMENTS
BEEKMAN, BRUCE E.
The Effect of Synthalin A on Blood Sugar and Pancreatic Alpha Islet Cells of the Fowl..... 708
HOFMANN, FREDERICK G.
Observations on *In Vitro* Adrenal Steroid Synthesis in the Albino Mouse 712
MIRSKY, I. ARTHUR, DANIEL DIENGOTT AND GLADYS PERISUTTI
The Hypoglycemic and Insulinase-Inhibitory Action of Some Plant Growth Regulators..... 715
BARKER, S. B.
In Vitro Response of Kidney Tissue Metabolism to Thyroxine..... 719
ENDOCRINOLOGY STUDY SECTION, NIH..... 722
AMERICAN CANCER SOCIETY..... 723
INTERNATIONAL ABSTRACTS OF BIOLOGICAL SCIENCES..... 723
INDEX..... 724

ISSUED MONTHLY FOR THE ENDOCRINE SOCIETY
BY CHARLES C THOMAS, PUBLISHER,
SPRINGFIELD, ILLINOIS

nual Subscription $15.00 Single Copies $1.50

A "SENSE OF WELL-BEING" IS A WOMAN'S PRIVILEGE

Every woman who suffers in the menopause deserves "Premarin."

Relief from distressing symptoms is promptly obtained and a "sense of well-being" is an extra benefit of therapy.

"Premarin" presents the complete equine estrogen-complex. Has no odor, imparts no odor. Available as tablets or liquid.

"PREMARIN"®

Conjugated estrogens (equine)

in the menopause and the pre- and postmenopausal syndrome

Ayerst

AYERST LABORATORIES • New York, N. Y. • Montreal, Canada

5644

ENDOCRINOLOGY

VOLUME 59 DECEMBER, 1956 NUMBER 6

Copyright © 1956 by the Endocrine Society

NEUROHYPOPHYSIS AND WATER METABOLISM IN THE TOAD, *BUFO BUFO* (L.)

C. BARKER JÖRGENSEN, K. G. WINGSTRAND AND PER ROSENKILDE

Laboratory of Zoophysiology, and Institute for Comparative Anatomy, University of Copenhagen, Denmark

SEVERAL attempts have been made to evaluate the role of the neurohypophysis in the water metabolism of amphibians by extirpation of the hypophysis (1, 2, 3, 4, 5, 6, 7). However, since it is technically difficult to extirpate the neurohypophysis only the adenohypophysis was removed together with the neurohypophysis. Such experiments give no information on the function of the neurohypophysis, for it is known that extirpation of the pars distalis of the hypophysis strongly influences the water balance. For example, the cutaneous effect of the neurohypophysial extracts is absent or greatly diminished if the pars distalis has been removed (8, 9, 6). Also, the antidiuretic effect of such extracts is reduced in frogs without the pars distalis (unpublished data).

In the present work we have investigated the water balance in toads after sectioning of the praeoptic-hypophysial tract. This operation often resulted in complete degeneration of the neural lobe (processus infundibuli), whereas the pars distalis remained intact.

The ability of operated and normal animals to control their water balance was tested by measuring the effect of dehydration on the diuresis and on the permeability of the skin to water. In order to see whether the hormone content of the gland diminished in response to dehydration we further measured the amount of water balance principle[1] in the neurohypophysis of normally hydrated and dehydrated toads.

Received March 30, 1956.

[1] The substance(s) responsible for the renal and cutaneous effects of amphibian neurohypophysial extracts is called the amphibian water balance principle. It is probably the homologue of the antidiuretic hormone of the mammalian pituitary gland (10, 11, 12, 13).

TECHNIQUE

Operations were made on urethane anaesthetized adult female toads of the species *Bufo bufo* (L.). The brain was exposed by removing part of the sphenoid. The praeoptic-hypophysial tract was cut just rostrally of the median eminence, where the fibers form a well-defined bundle in the floor of the third ventricle (Fig. 1). Care was taken not to

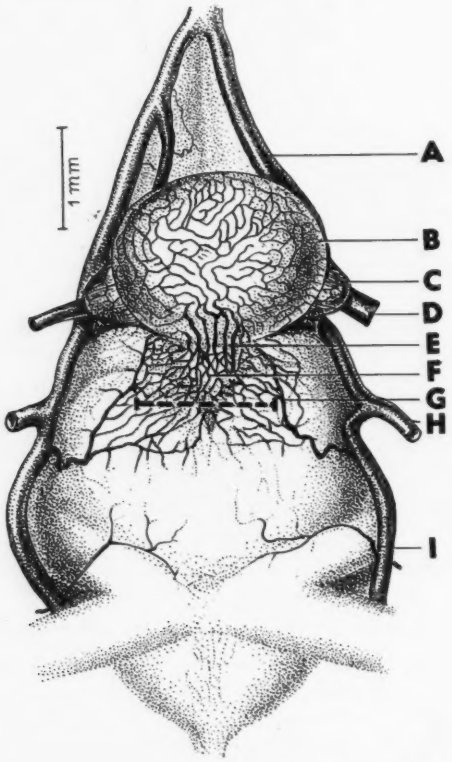


FIG. 1. Hypothalamic-hypophysial region of a toad. Ventral view. Dotted line indicates site of sectioning. Drawn from a dissected specimen, injected with India ink. A: Radix post. carotis, B: Pars distalis, C: Pars intermedia + neural lobe, D: Vein, E: Portal vessels, F: Median eminence, G: Artery to median eminence, H: Carotis interna, I: R. ant. carotis.

disturb the hypophysial portal circulation, which is important for the normal functioning of the pars distalis (14). The operation was completed by replacing the parasphenoid and closing the wound with two stitches. The functional state of the pars distalis after operation was judged from the appearance of the skin of the toads. Hypofunction results in various degrees of enhanced cornification of the skin and failure to shed the old cornified layers. If the pars distalis is absent or not functioning the animals die within a few weeks.

Most operated toads as well as controls were afterwards killed and the condition of

the hypothalamo-hypophysial region was determined by histological examination. The heads were fixed in Bouin, the brains were dissected out, embedded in paraffin and cut in serial sections at 8 μ. The slides were stained in Gomori's haematoxylin and phloxine after permanganate oxidation and sulphite reduction (15).

In one series of experiments the volume of the neural lobe was measured. Every fourth section was projected on paper and the neural lobe tissue drawn, cut out, and weighed. The sinusoidal capillaries are thus not included in the volume, nor is the median eminence.

Rates of urine formation were measured in toads upon transfer from water to the saturated atmosphere of glass vessels lined with moist filter paper. The toads were kept in wire cages and thus not in direct contact with the paper because they may absorb water from it. At suitable intervals the bladder was emptied by catheterization and the animals weighed. The decrease in weight shows approximately the amounts of urine produced, as long as the renal loss of water is large compared with the small weight loss due to evaporation from the skin.

For dehydration the toads were placed in glass beakers covered by a wire net. Water was lost at a rate corresponding to some 10% weight loss in 24 hours.

Assay of water balance principle of neurohypophysial extracts was made by measuring the effect of the extracts upon the rate of water uptake in toads. The glands were ground with sand in a few drops of Ringer, and extracts were made by boiling for five minutes with a few cc. of 0.25% acetic acid. The extracts were not filtered. Dilutions were made with tap water.

The assay was made in the following way. Extracts of glands from hydrated and dehydrated toads of the same size were diluted to contain equal fractions, generally one, two or three hundredths of a gland, in 0.25 cc., which was the quantity injected into each toad. The dilution was adjusted so that injection of 0.25 cc. gave a pronounced but clearly submaximal effect. Thirty-two healthy male or female toads were injected for each assay. They were placed singly in glass vessels with water to the back of the neck. The urinary bladder was emptied by catheterization before the extract was given subcutaneously. The toads were then gently dried with a soft cloth, weighed, and put back into the water. After an hour they were dried and weighed again, and the weight increase was used as a measure of the rate of water uptake. The test toads were divided into four groups of 8 animals. The groups were injected alternately with the two extracts to be compared. When the effect of the injection had vanished, usually after five to six hours, the extracts were administered to the groups in the reversed order, so that every one of the 32 toads received injections of both extracts on the same day. In experiments with that many toads some might be moulting and therefore have abnormally high values of water uptake (16, 17) and occasionally one might void urine before the final weighing was made. Values from such toads were discarded. However, 25–30 pairs of values of water uptake were generally obtained in an assay.

The rate of water uptake produced by the active extracts was expressed in per cent of the rate after injection of tap water. The response to tap water which differs little from the normal rate of water uptake (12), was measured both on days before and after the assay of the extracts.

In separate experiments the response of the test animals was measured to various doses of extract. From the dose-response relationship differences in activity of neurohypophyses from hydrated and dehydrated toads could be estimated.

RESULTS

The effect of destruction of the praeoptic-hypophysial tract was investigated in two series of toads:

I. The first series included 11 operated and 6 control toads, all females. The rate of urine formation upon removal from water was measured two weeks after operation, and the effect of dehydration on water uptake was measured 3 and 6 weeks after operation. Seven operated animals and 4 controls were used for histological examination 7 weeks after the operation, which took place in October.

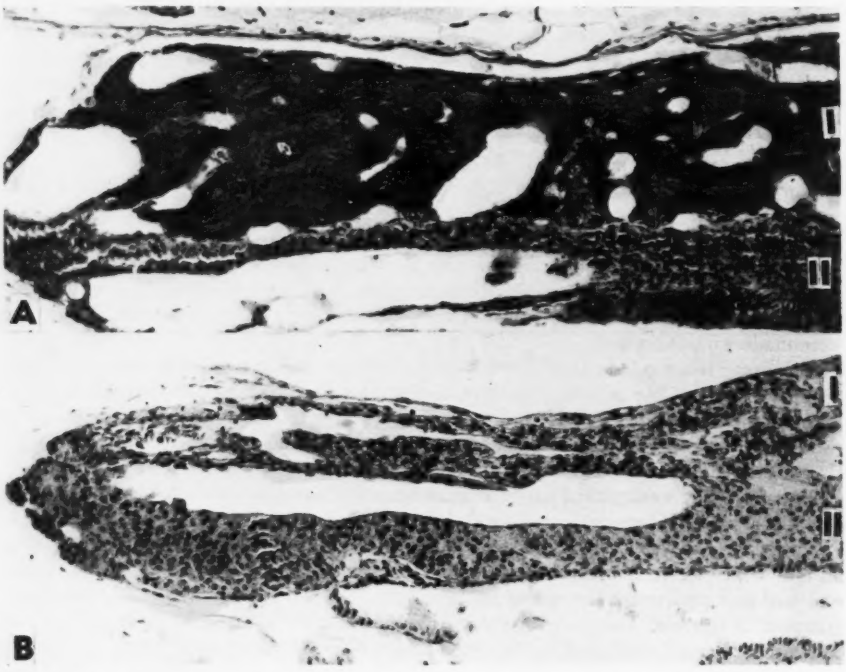


FIG. 2. Cross section of a normal neural lobe (A) with its content of dark stained neurosecretory material, and of a degenerated neural lobe (B) .7 weeks after sectioning of praeoptic-hypophysial tract. I = neural lobe, II = pars intermedia. Gomori's haematoxylin and phloxin, × 110.

II. The second series included 5 operated and 6 normal toads, all females. The rate of water uptake after dehydration was determined 14 weeks after operation, which took place in December. The animals were used for histological examination 18 weeks after operation.

Histological findings. In the first series the operation was successful in 6 animals, with the neural lobe showing signs of degeneration. In the one unsuccessfully operated toad the praeoptic-hypophysial tract had been only partially sectioned. Whereas the neural lobe of the controls was filled with neurosecretory substance stained by Gomori's haematoxylin, that of the successfully operated animals contained very little or practically no such substance (Fig. 2). The volume of the neural lobe had diminished from

0.121 mm.^{3} and 0.154 mm.^{3} per 100 gm. body weight in the controls to 0.034, 0.039, 0.044, 0.051, 0.053 and 0.054 mm.^{3} per 100 gm. body weight in the operated animals. In all the operated toads there was a marked accumulation of neurosecretory substance rostrally to the cut, and some fibers of the praeoptic-hypophysial tract were distended and filled with the substance for a considerable distance in the direction of the praeoptic

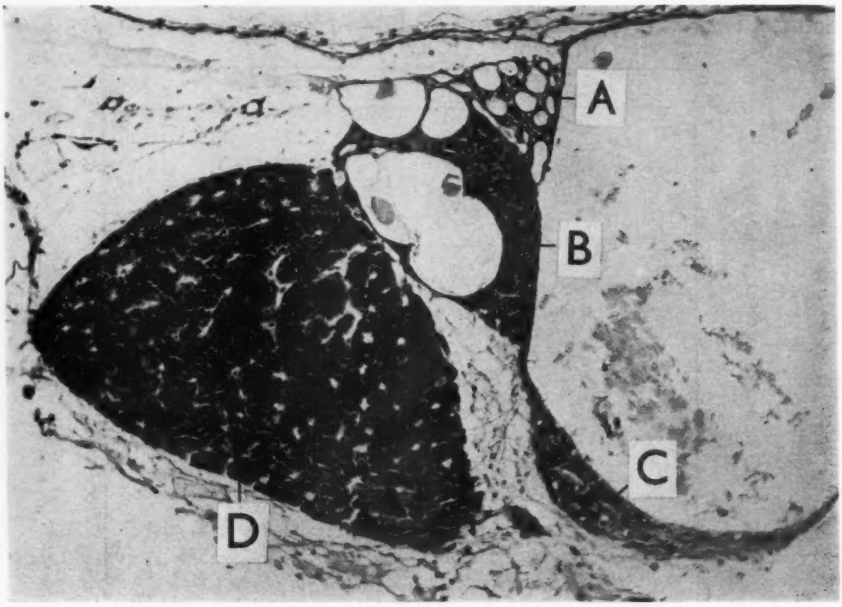


FIG. 3. Median section of a toad hypophysis 18 weeks after sectioning of praeoptic-hypophysial tract. A = degenerated neural lobe, B = pars intermedia, C = median eminence, D = pars distalis, × 120.

nucleus. The cells of the praeoptic nucleus still contained neurosecretory substance, and in some specimens they looked "swollen" with large pale nuclei and abundant plasma. Nuclear pycnosis or disintegration of cells was not observed with certainty. In three specimens there was central necrosis in the pars distalis. Two of these specimens showed signs of deficient function of the pars distalis already when alive.

In the second series two of the 5 operated toads showed almost complete degeneration of the neural lobe. The organ was reduced to thin membranes between the vessels (Fig. 3). The three remaining toads showed reduction in size of the neural lobe, but considerable amounts of neurosecretory substance were present. Some bundles of neurosecretory fibres ran laterally to the operation wound to the neural lobe. These bundles appeared to be the result of regeneration and it is known that fibres of the praeoptic-hypophysial tract have a considerable ability to regenerate (18).

There was still some accumulated neurosecretory substance on the proximal side of the operation wound in these animals 18 weeks after operation. The cells in the dorsal part of the praeoptic nucleus contained some neurosecretory substance. The ventral part of the nucleus, in the very praeoptic recess, appeared to be degenerating, and numerous cells had so little plasma, that little but the cell nucleus was seen.

The effect of dehydration on the rate of water uptake in the two series of animals is shown in Figures 4 and 5.[2] From Figure 4 it may be seen that

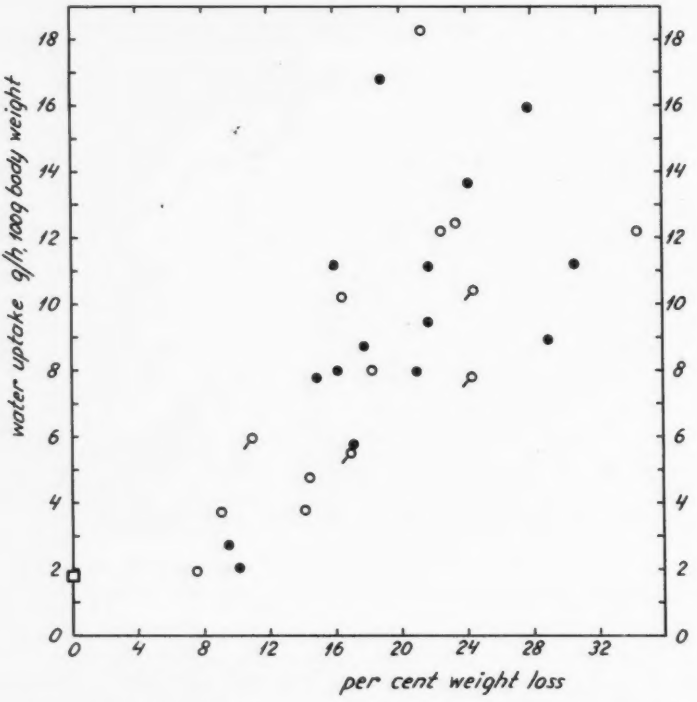


FIG. 4. Effect of dehydration on rate of water uptake: ○ three months after sectioning of praeoptic-hypophysial tract. ℘ indicates that the neurohypophysis was completely degenerated. ● controls. Temp. 24–25° C.

the toads responded to dehydration, three months after sectioning of the praeoptic-hypophysial tract, as strongly as the toads with an intact neurohypophysis. Similar results were obtained on toads three and six weeks after the operation, but these experiments were complicated by the impaired function of the pars distalis in some animals (Fig. 5). It was found that all the low values of water uptake in the operated toads belonged to

[2] There was no difference in the rate of water uptake in normally hydrated operated and control toads.

animals with defective function of the pars distalis. The toads which lacked neurosecretory material in the neural lobe were not inferior to the normals in ability to increase water uptake. No difference was found between the results obtained three and six weeks after operation.

The effect of sectioning the praeoptic-hypophysial tract on the oliguretic response in toads transferred from water to a saturated atmosphere is shown in Table 1A, which only includes values from undisturbed toads. In water the rate of urine flow and water uptake were the same because the

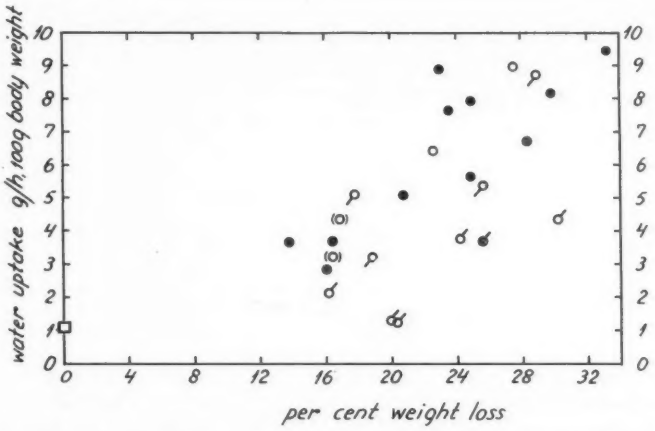


FIG. 5. Effect of dehydration on rate of water uptake: ○⊗♂♀(○) 3 and 6 weeks after sectioning of praeoptic-hypophysial tract; ⊗ operated toads not microscopically examined; ♂ toads with hyperkeratinization of the skin and abnormal moulting indicating impaired function of the pars distalis; ♀ toads with practically no neurosecretory substance in the neurohypophysis. (○) one toad with only partial sectioning of the praeoptic-hypophysial tract. ● controls. Temp 18° C.

toads maintained a constant weight. Water uptake and thus diuresis was about 1.1 cc./hr. per 100 gm. body weight both in operated and control toads. Within 24 hours in a humid atmosphere the diuresis fell to 1–2% of the value in water, with no significant difference between operated and control toads. As mentioned, the histological examination revealed complete sectioning of the praeoptic-hypophysial tract in all but one of the examined toads of this series. The unsuccessfully operated toad is not included in Table 1 A, whereas three toads not histologically examined are included.

There was a considerable influence on the diuresis when the toads seemed excited. Table 1B is arranged so that values from periods of restlessness are in one group, and those from periods of quiescence in the other. The same toad often contributed to both groups. It is seen that restless toads, that is toads which tried hard to escape from the glass vessels, had much

greater diuresis than the quiet toads. In the first three hours out of the water the urine flow of the restless toads was even consistently higher than the average diuresis in water. At the end of 24 hours the quiet toads, operated as well as not operated, had lost 1.4–4.7% of the initial weight against 5.9–9.4% in toads with one or more periods of restlessness.

Even when we were unable to demonstrate any significance of the neurohypophysis in producing oliguria and increased permeability to water in dehydrated toads, it was still possible that water balance principle was

TABLE 1. DIURESIS IN TOADS IN WATER AND UPON TRANSFER TO A SATURATED ATMOSPHERE. A. DIURESIS IN UNDISTURBED NORMAL TOADS AND IN UNDISTURBED TOADS AFTER SECTIONING OF THE PRAEOPTIC-HYPOPHYSIAL TRACT. B. DIURESIS IN UNDISTURBED AND IN DISTURBED TOADS, BOTH GROUPS INCLUDING OPERATED AS WELL AS UNOPERATED ANIMALS

		Diureses in cc./hr., 100 gm. body weight			
		In water	After removal from water		
			1–3 hrs.	4–6 hrs.	7–24 hrs.
A	Controls	1.12 ±0.08	0.88 ±0.14	0.26 ±0.13	0.01
	Operated toads	1.07 ±0.06	0.80 ±0.15	0.47 ±0.15	0.02
B	Undisturbed toads		0.80 ±0.10	0.38 ±0.10	0.023 ±0.007
	Disturbed toads		1.78 ±0.14	1.07 ±0.03	0.118 ±0.023

liberated from the neurohypophysis of normal toads during dehydration. In mammals it is known that dehydration may markedly reduce the content of antidiuretic hormone in the neurohypophysis (19, 20). In frogs dehydration was found to lower the oxytocic activity of the neurohypophysis (21). In toads excessive dehydration practically emptied the gland of antidiuretic substances, tested on rats (22). We therefore investigated the possible effect of dehydration on the content of the water balance principles of the toad neurohypophysis. Male toads were dehydrated to a loss of about 25–30% of their initial weight and were kept dehydrated for two to seven days. Extracts were made of the pooled neurohypophyses from each group of three to six dehydrated toads. Extracts were further made of the glands from equal numbers of normally hydrated toads of the same size. From Table 2 it is seen that dehydration for more than three days resulted in a statistically significant fall in the content of water balance principle of the neurohypophysis. The glands of the dehydrated toads exerted only about $\frac{2}{3}$ to $\frac{1}{4}$ of the water balance activity of glands from normally hydrated toads.

DISCUSSION

Successful sectioning of the praeoptic-hypophysial tract in the toad resulted in degeneration of the neural lobe of the pituitary, whereas the pars distalis remained functional. The operation did not influence the increase in skin permeability caused by dehydration, nor did it interfere

measurably with the oliguria which sets in when the toads are deprived of water. The oliguretic effect of water loss was measured two weeks after sectioning of the tract, at a time when the neurohypophysis was degenerating and most probably not functioning. In the dog the hypothalamo-hypophysial tract ceased participating in the regulation of the water metabolism immediately upon sectioning (23). It is reasonable to assume that this also applies to the toad because of the great similarities in the structure of the hypothalamo-neurohypophysial systems in mammals and amphibians.

TABLE 2. EFFECT OF DEHYDRATION ON CONTENT OF WATER BALANCE PRINCIPLE OF NEUROHYPOPHYSIS OF TOADS

Percentage increase in rate of water uptake in toads after injection of extract of 1/300 or 1/100 neurohypophysis from		Statistical significance of difference in water balance activity of neurohypophyses from hydrated and dehydrated toads	Water balance activity of neurohypophysis of dehydrated toad in percent of the control	Duration of dehydration in days
Hydrated toads	Dehydrated toads			
199 ± 11.5	192 ± 10.3	0.6 < P < 0.7	80	2
174 ± 10.3	156 ± 7.7	P = 0.17		2
232 ± 16.3	193 ± 15.1	P = 0.1	39	3
181 ± 7.6	156 ± 6.4	0.01 < P < 0.02		3
236 ± 10.5	202 ± 10.2	0.02 < P < 0.03	57	4
203 ± 10.9	172 ± 8.6	P = 0.03	57	7

Dehydrated frogs were found to regain weight in water at a slower rate if the hypophysis (including the pars distalis) had been extirpated (21). The experiments were carried out on the day following operation. It was concluded that the presence of the neurohypophysis speeded the rate of rehydration. However, the experiments are not entirely conclusive as regards the function of the neurohypophysis, because the controls were non-operated frogs and not animals with extirpated pars distalis. Presently, therefore, it cannot be decided whether frogs and toads are different with respect to the importance of the neurohypophysis in the water metabolism. Anyhow, the neurohypophysis is probably not solely responsible for the increased permeability to water in dehydrated frogs because both in frogs and toads dehydration is much more effective in increasing the permeability to water than are neurohypophysial extracts (24).

SUMMARY

The role of the neurohypophysis in the water metabolism of the toad *Bufo bufo* (L.) was investigated by sectioning of the praeoptic-hypophysial tract. The neural lobe of the neurohypophysis degenerated after this operation. Seven weeks after the sectioning the lobe was reduced to about one third of the normal size and it contained little or no neurosecretory substance stainable with Gomori's haematoxylin.

Two weeks after the operation at a time when the neural lobe was

probably not functioning the toads showed a normal oliguretic response to water deprivation. When undisturbed operated and control toads were transferred from water to a saturated atmosphere the diureses in both groups fell within 24 hours to 1–2% of the values in water. Both normal and operated toads showed increased rates of urine flow during periods of restlessness.

Dehydration increases the permeability of the amphibian skin to water. The effect of dehydration on water uptake in water was measured in toads 3, 6 and 14 weeks after operation. Even toads with completely degenerated neural lobes responded as strongly to dehydration as the controls.

Dehydration for three or more days corresponding to a weight loss of 25–30% reduced the water balance activity of the neurohypophysis to about one half of normal, or less. Apparently, therefore, water balance principle is liberated from the neurohypophysis during dehydration, but the cutaneous and renal responses to dehydration have so far been found to be independent of neurohypophysial activity in the toad.

REFERENCES

1. Braun, R.: *Zool. Jahrb. Allg. Zool. u. Physiol. Tiere* **64**: 332. 1953.
2. Jungmann, P. and H. Bernhardt: *Zeitschr. Klin. Med.* **99**: 84. 1923.
3. Pasqualini, R. Q.: *Papel de la hipofisis en le regulacion de la diuresis.* Buenos Aires. Diss. 1938.
4. Pohle, E.: *Arch. ges. Physiol.* **182**: 215. 1920.
5. Rey, P.: *Recherches expérimentales sur l'économie de l'eau chez lez Batraciens.* Paris. Diss. 1937.
6. Steggerda, F. R. and M. E. Jones: *Amer. J. Physiol.* **112**: 397. 1935.
7. Tschernikoff, A.: *Arch. ges. Physiol.* **212**: 187. 1926.
8. Chen, G., F. K. Oldham and E. M. K. Geiling: *Proc. Soc. Exper. Biol. Med.* **52**: 108. 1943.
9. Levinsky, N. G. and W. H. Sawyer: *Endocrinology* **51**: 110. 1952.
10. Heller, H.: *Biol. Rev.* **20**: 147. 1945.
11. Heller, H.: *Experientia* **6**: 368. 1950.
12. Jörgensen, C. B.: *Acta Physiol. Scand.* **22**, Suppl. **78**: 1. 1950.
13. Sawyer, W. H., D. F. Travis and N. G. Levinsky: *Amer. J. Physiol.* **163**: 364. 1950.
14. Houssay, B. A.: *Quart. Rev. Biol.* **24**: 1. 1949.
15. Gomori, G.: *Amer. J. Path.* **17**: 395. 1941.
16. Ewer, R. F.: *J. Exp. Biol.* **28**: 369. 1951.
17. Jörgensen, C. B.: *Acta Physiol. Scand.* **18**: 171. 1949.
18. Wingstrand, K. G., C. B. Jörgensen and P. Rosenkilde. Unpublished.
19. Diamond, M. C.: *Endocrinology* **58**: 461. 1956.
20. Hild, W. and G. Zetler: *Pflügers Arch.* **257**: 169. 1953.
21. Levinsky, N. G. and W. H. Sawyer: *Proc. Soc. Exp. Biol. Med.* **82**: 272. 1953.
22. Tramezzani, J. H. and J. V. Uranga: *Rev. Soc. argent. Biol.* **30**: 148. 1954.
23. O'Connor, W. T.: *Quart. J. Exp. Physiol.* **37**: 1. 1952.
24. Jörgensen, C. B. and P. Rosenkilde: *Biol. Bull.* **110**: 306. 1956.

THE EFFECT OF INJECTED THIOUREA ON THE DEVELOPMENT OF SOME ORGANS OF THE CHICK EMBRYO[1]

ALEXIS L. ROMANOFF AND HANS LAUFER[2]

Agricultural Experiment Station, Cornell University, Ithaca, New York

THE thyroid gland of the chick embryo is sensitive to antithyroid compounds, such as thiourea and methyl and propyl thiouracil. The presence of a goitrogen in the egg, whether deposited there from maternal dietary sources (1) or injected directly (2, 3, 4, 5), results in retarded growth, incomplete retraction of the yolk sac, delayed hatching, increased mortality and thyroid hypertrophy. Another effect is atrophy of the adrenal cortex accompanied by hypertrophy of the medulla (6). Yushok (7) found that the liver weighs much less than normal in hypothyroid embryos, whereas the heart and kidneys exhibit relatively smaller decreases in size, and the brain little or none; also, he noted a considerable reduction in the cytochrome-*c* content of the organs and the entire body.

The investigation described below was undertaken to supplement the above results previously obtained by Yushok (7) in this laboratory. It seemed desirable to determine whether or not the weights (wet and dry) of additional organs (such as the adrenals and gonads) is affected when thiourea is administered in different amounts and at various times during incubation. In order to provide a sound basis of comparison with normal development, it was decided to continue observations throughout the period of retardation following the twenty-first day of incubation. Some information was also sought regarding the postnatal survival and development of chicks subjected to the influence of thiourea before hatching.

EXPERIMENTAL METHODS AND MATERIALS

Eggs of White Leghorn hens (*Gallus gallus d.*) were obtained from a commercial breeder and from Cornell University stock. A total of 631 fertile eggs were used. They were selected for good shell texture and for uniformity of size (mean weight, 62.0 gm.) and were incubated in a forced-draft incubator at the temperature (37.5° C) and relative humidity (about 60%) which produce maximum hatchability (8). The eggs were turned automatically every two hours and were candled at intervals of twenty-four hours.

The albumen was chosen as the site of injection because a needle inserted into this component of the egg is least likely to damage embryonic or extraembryonic tissues. The ingestion of introduced thiourea is assured, for the albumen duct becomes patent at a time that coincides approximately with the beginning of thyroid function, and the

Received May 16, 1956.

[1] This study was supported in part by a W. D. James Fellowship.

[2] Research Assistant in Chemical Embryology.

embryo thereafter swallows the albumen. Thiourea injected earlier is presumably absorbed via the chorioallantoic circulation in the same manner as trypan blue (9).

Following the technique of Yushok (7), injections were made into the albumen at the small end of the egg in a region shown by candling to be free of major allantoic blood vessels. This region was outlined with a pencil, bathed in 70% ethyl alcohol, and covered with a film of collodion. With the egg held horizontally to prevent the entrance of air, the egg shell was pierced with a flamed dissecting needle of fine bore while the collodion film was still moist. The hole thus made was immediately employed for the injection (with a sterilized hypodermic needle, No. 26) of 0.5 ml. of warmed (37.5° C), previously autoclaved solution, consisting of chicken Ringer's solution (10) with added thiourea. Upon withdrawal of the hypodermic needle, another drop of collodion was applied and the eggs were returned to the incubator. As indicated in the following descriptions of the experiments and their results, some embryos were allowed to incubate until their development terminated in death, detectable by candling, whereas other were sacrificed at desired ages. Controls consisted of uninjected eggs and eggs injected with saline, which was found to be without effect on development.

The wet tissues were rolled on filter paper to remove excess moisture and were then weighed on either a Sartorius or an analytical balance, depending upon the amount of tissue. Dry weights were determined after the tissues had been dehydrated for at least twenty-four hours at 55° C in a continuous vacuum of about twenty-five inches.

RESULTS AND DISCUSSION

A. *The influence of different doses of thiourea*

Reports are somewhat at variance regarding the correlation between the dosage of thiourea and the response of the chick embryo (2, 3, 5). To compare the effect of different doses, eggs were injected with 2, 3, 5, or 10 mg. of thiourea on the eleventh day of incubation, when the thyroid is known to be functional (11).

One lot of 144 eggs injected with the above amounts was incubated until the embryos either hatched or died. The mortality rate and the prolongation of the developmental period were both in roughly direct proportion to the quantity of thiourea administered, as the following tabulation indicates:

	Amount of thiourea injected (mg.)				
	0	2	3	5	10
Total mortality (per cent)	7	25	85	100	100
Length of incubation period (days)	21	24–28	24–28	26–31	29–31

A second lot of 49 injected eggs was incubated for only twenty-one days, at the end of which time the embryos were sacrificed and their body and organ weights determined. Figure 1 shows that 3 mg. of thiourea produced the greatest reduction in the wet weight of the liver and the entire embryo. On the other hand, the smallest dose (2 mg.), although of least effect on body and liver weight, caused the largest increase in the wet weight of the thyroid. The changes in the dry weights of the liver, thyroid, and adrenals followed the same trends as the changes in the wet weights, but the devia-

tions from normal were relatively smaller; hence it was apparent that the water content of all the tissues was increased. The adrenals and gonads were not significantly affected by the administration of thiourea.

The observed smaller effect of the larger dosages of thiourea on body and other weights is consistent with similar results obtained by Adams and Bull (3). The possibility that some of the thiourea was made unavailable by precipitation is suggested by two circumstances. First, a precipi-

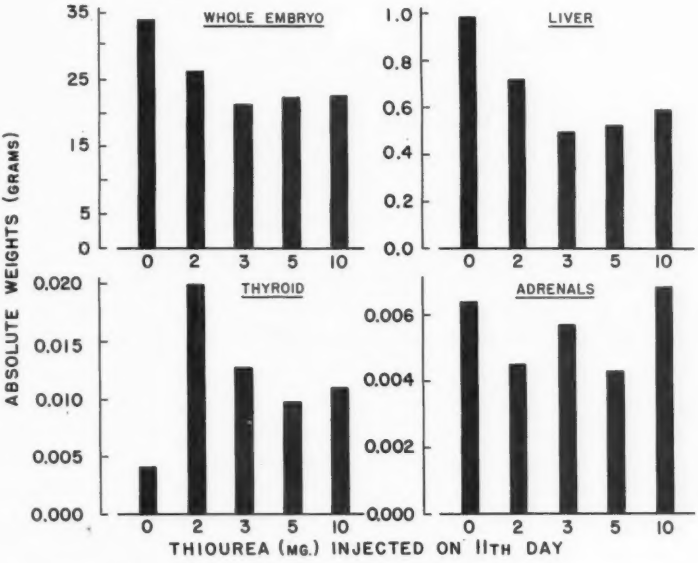


FIG. 1. The weight of the entire chick embryo and several of its organs on the twenty-first day of incubation, as influenced by the injection of various amounts of thiourea on the eleventh day.

tate forms immediately when a 10% solution of thiourea is layered over egg albumen in a test tube; second, a small white mass was present in many of the unhatched eggs at the site of injection.

B. *The influence of the time of injection*

Antithyroid compounds have usually been introduced into the chicken egg either before incubation or during the period (8 to 11 days) when the thyroid is becoming physiologically active, as shown by its ability to concentrate the radioactive isotope, I^{131} (11, 12, 13, 14). Little is known of the effect of administering antithyroid substances during the first week of development, except for the observation (5) that 2.5 mg. of thiourea injected into the yolk sac at 0 or 48 hours failed to produce any deviation from normal embryonic body weight by the eighth day. In order to in-

vestigate the possible relationship between the effect of this drug and the time of its administration, thiourea was injected into a different group of eggs on each successive day from 0 to 18 days of incubation. Each egg received a single 5-mg. dose. Altogether, 182 eggs were used. They were returned to the incubator until embryonic development terminated.

All the embryos died except for eight, which hatched on the twenty-first day from eggs injected at 18 days. About 18% of all the remaining embryos died before the twenty-first day and were discarded; over half

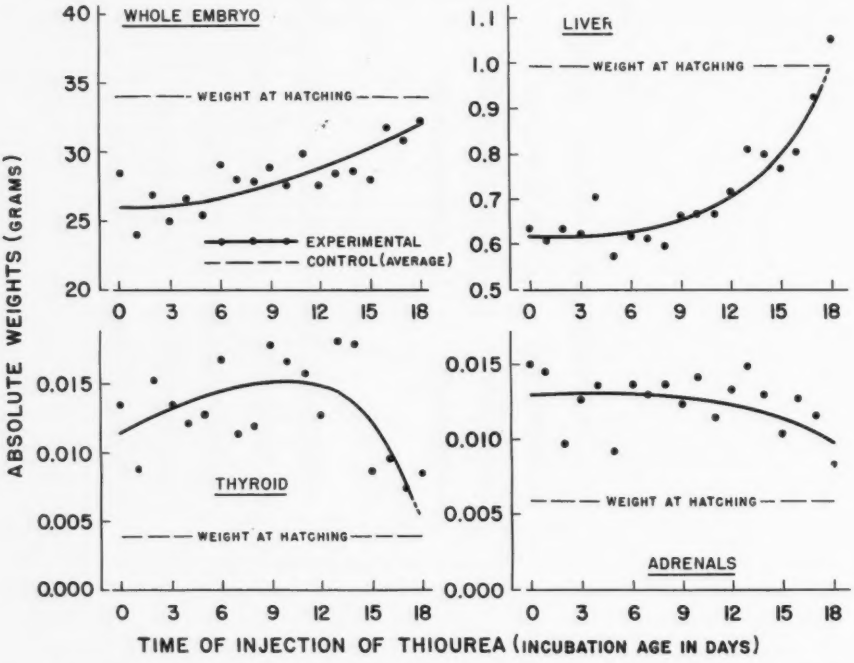


FIG. 2. The wet weight of the chick embryo and some of its organs at the end of development, as affected by 5 mg. of thiourea injected into the egg at different times during the incubation period.

the premature deaths occurred at 6 to 9 days and more than three-fourths of them were among embryos treated at 0 to 7 days. The rest of the embryos died after prolonged incubation periods, which ranged from 22 to 35 days in length but averaged slightly over 28 days. Among the embryos injected prior to 14 days there was no particular correlation between the average length of the developmental period and the duration of exposure to thiourea. The average incubation period gradually shortened to 26.3 days as the age at the time of injection increased from 14 to 17 days.

The changes in total weight and in the weights of the organs are shown in graph form in Figure 2. As the curve for thyroid weight indicates,

thiourea produced maximum thyroid enlargement when given at 9 to 12 days but seemed to cause somewhat less hypertrophy when injected prior to this period. From this finding, it might be inferred that some of the injected thiourea was unavailable during the first week of incubation, before it could affect the thyroid. However, injections given at any time before the period of initial thyroid function resulted in maximal changes in the weight of the liver and in the weight of the adrenals (which were greatly enlarged). The effect on these organs suggests either: 1, that an equivalent degree of thyroid deficiency was produced by all early injections (as would be the case if the injected thiourea did not take effect until the thyroid became functional); or 2, that the differences in thyroid deficiency associated with the observed differences in thyroid weight were of insufficient magnitude to be reflected by other body tissues and organs.

When thiourea was given after the onset of thyroid activity, the effect on the thyroid, liver, and adrenals was directly proportional to the length of time the drug was allowed to exert its influence, that is, there was an inverse relationship between response and age at the time of injection. Total body weight seemed to show this relationship in embryos treated on or after the sixth day.

In general, it can be concluded that the period from the eighth to tenth days, inclusive, is the optimum time to inject thiourea into the chicken egg, and that little or nothing is to be gained by injecting it earlier.

Table 1 summarizes the results of wet and dry weight determinations made on the embryos from the eggs injected on the eleventh day. It reveals that the thyroid suffered a far greater deviation from normal than the other organs, and that the adrenals were affected much more than the liver. The data for the gonads were rather inconclusive, since the wet weight of the testes increased and that of the ovary diminished. The table also shows that the water content of all organs examined was elevated by at least 15% regardless of whether their respective wet weights were higher or lower than normal.

In Table 2, wet and dry weights are expressed as grams per 100 gm. of total embryonic weight. The same general relationships exist.

C. *Development of hypothyroid embryos after the twenty-first day*

Eggs were injected with 5 mg. of thiourea on the eleventh day of incubation. By sacrificing embryos when necessary, it was possible to determine body and organ weights daily from the twenty-first to the thirty-first day, inclusive, of the prolonged developmental period.

Body weight was equivalent on the twenty-first day to the normal 18-day value (15) and increased until the twenty-eighth day, when it corresponded to the normal 20-day value (Fig. 3). The growth curve began to level off on the twenty-sixth day, after which time deaths were most frequent. Comparison of the growth curves for hypothyroid embryos and normal chicks, both embryonic and hatched (minus yolk sac), shows that

TABLE 1. ABSOLUTE AND RELATIVE WEIGHTS OF LIVER, THYROID, ADRENALS AND GONADS OF CHICK EMBRYO AT THE TIME OF DEATH (EXPERIMENTAL) AND HATCHING (CONTROLS)

Observations	Experimental[1]		Control[2]		Deviation of experimental from control
	Number	Average value	Number	Average value	
Absolute weights:		gm.		gm.	per cent
Whole embryo					
Wet	53	28.8	109	34.2	− 15.8
Liver					
Wet	54	0.639	105	0.948	− 32.6
Dry		0.156		0.328	− 52.3
Thyroid					
Wet	53	0.0155	109	0.0037	+318.9
Dry		0.0031		0.0012	+158.3
Adrenals					
Wet	59	0.0124	104	0.0061	+103.3
Dry		0.0027		0.0020	+ 35.0
Testes					
Wet	27	0.0087	61	0.0061	+ 42.6
Dry		0.0018		0.0020	− 10.0
Left ovary					
Wet	31	0.0053	41	0.0056	− 5.3
Dry		0.0016		0.0021	− 23.8
		per cent		per cent	per cent
Water contents:					
Liver		75.6		65.4	+ 15.6
Thyroid		80.0		67.6	+ 18.3
Adrenals		78.2		67.2	+ 16.4
Testes		79.4		67.2	+ 18.2
Left ovary		79.8		62.5	+ 27.7

[1] Five milligrams of thiourea injected into the albumen of each egg on the 11th day of incubation and examined at the time of death, about 28 days.
[2] Combined values of all controls.

there is a continually greater divergence between the two curves after the eleventh day (*cf.* Fig. 3).

The weight of the liver on the twenty-first day was approximately the same as the normal 17- or 18-day weight (16) and continued to increase

TABLE 2. WET AND DRY WEIGHT OF ORGANS PER 100 GRAMS OF WET WEIGHT OF EMBRYO IN 28-DAY EXPERIMENTAL[1] AND 21-DAY CONTROL CHICK EMBRYOS

Observations	Experimental		Control		Deviation of experimental from control	
	Wet	Dry	Wet	Dry	Wet	Dry
	gm./100 gm.	gm./100 gm.	gm./100 gm.	gm./100 gm.	per cent	per cent
Liver	2.22	0.543	2.77	0.959	− 20.0	− 44.2
Thyroid	0.057	0.010	0.011	0.003	+441.5	+209.4
Adrenals	0.048	0.010	0.018	0.005	+172.2	+ 83.3
Testes	0.029	0.006	0.017	0.005	+ 65.7	+ 11.1
Left ovary	0.018	0.005	0.015	0.005	+ 20.4	− 6.1

[1] See footnote of table 1.

until the thirtieth day; by this time, however, it attained only the normal 19-day value (Fig. 3). During the same period, the dry weight of the liver showed a parallel increase. The rapid increase in the weight of normal chicks' livers during the first nine postembryonic days (*cf.* Fig. 3) proved to be due to a relatively greater accumulation of water than of dry substance.

The thyroid in the experimental embryos was about three times as heavy as normal on the twenty-first day. Its wet weight declined after the twenty-eighth day and eventually approached that of the thyroid of normal chicks at term (Fig. 3); dry weight underwent a parallel decrease. The

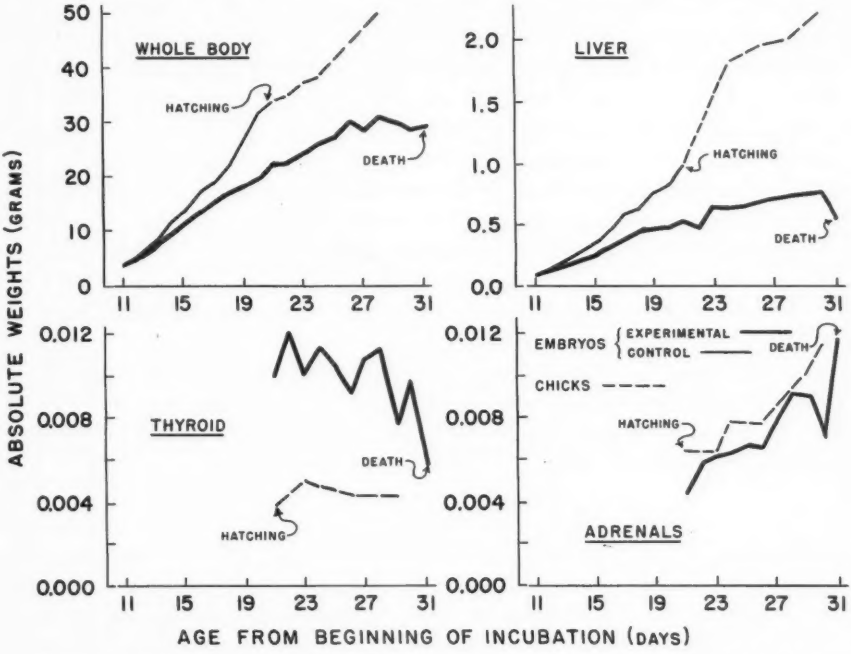


FIG. 3. The growth rate of the body and some of the organs of the chick embryo after the injection of 5 mg. of thiourea on the eleventh day of incubation. Normal curves are included for comparison.

thyroids of normal chicks increased very slightly in weight during the first week after hatching and had a lower water content than those of the thiourea-treated embryos.

The weight of the adrenals, wet or dry, was slightly less than normal at twenty-one days but increased approximately threefold by the thirty-first day, when it equaled that of the adrenals of the nine-day-old control (hatched) chicks (Fig. 3). Adrenal hypertrophy thus appeared to be a late effect.

Gonad weight was very variable and showed no definite trend.

D. *The postembryonic survival and development of hypothyroid chicks*

Eighteen chicks hatched from eggs that had been injected with 2 or 3 mg. of thiourea on the eleventh day of incubation. The yolk sac was incompletely retracted in six chicks. Five of the chicks (27.8%) died shortly, most of them before the fourth postembryonic day. Mortality among the control chicks was only 3.7%.

The ten surviving female chicks were observed until six weeks old, when they were sacrificed. (The males were excluded to avoid the influence of sex on body weight.) At hatching, the average live weight of these chicks was 42.0 gm., whereas that of the female control chicks was 44.7 gm. On the fourth day, both groups of chicks had the same average weight of about 50 gm.; thereafter, the experimental chicks were heavier than the controls, probably because of the greater accumulation of body fat that characterizes thyroid-deficient birds (17). Their advantage on the nineteenth day was as much as 25 gm. This advantage, however, was lost by the end of the sixth week, when average weight was the same for both groups of chicks.

The weights (wet and dry) of the liver and the adrenals were also equalized in the two groups of chicks by the end of the sixth week. At this time, the thyroid (wet or dry) of the treated chicks weighed 172% of normal; that is, much of the hypertrophy had disappeared.

The rate of feathering and growth of the comb were not appreciably different in the two groups of chicks.

It thus appears that hypothyroidism induced as in this experiment lowers the postembryonic viability of chicks but is largely overcome by the sixth week of life, when a lingering hypertrophy of the thyroid is the only grossly visible abnormality.

SUMMARY

Thiourea, in amounts varying from 2 to 10 mg., was injected into the albumen of chicken (*Gallus gallus d.*) eggs at different stages of incubation. The embryos were sacrificed or were permitted to develop until they hatched or died; the hatched chicks were raised to 6 weeks of age. Observations showed that the administration of thiourea resulted in prolongation of embryonic development by 3 to 10 days beyond the normal period of 21 days and increased prenatal mortality up to 100%, as compared with 25% mortality among the controls. The effects were, in general, proportional to the amount of drug administered. In all cases the thyroid was markedly hypertrophied, the weight of the organ increasing by as much as 300% during the embryonic period but dropping to 72% above normal during the postembryonic period. There was some increase in the weight of the adrenals and testes, a considerable decrease in the weight of the liver, and an irregular decrease in ovarian weight. The percentage of water was elevated in all the observed organs of the experimental embryos.

REFERENCES

1. ANDREWS, F. N. AND E. E. SCHNETZLER: *Endocrinology* **37:** 382. 1945.
2. GROSSOWICZ, N.: *Proc. Soc. Exp. Biol. Med.* **63:** 151. 1946.
3. ADAMS, A. E. AND A. R. BULL: *Anat. Rec.* **104:** 421. 1949.
4. ADAMS, A. E. AND J. M. BUSS: *Endocrinology* **50:** 234. 1952.
5. VIDAL, A.: *Ann. Endocrinologie* **13:** 982. 1952.
6. KINGSBURY, J. W., S. L. EMERY AND A. E. ADAMS: *Endocrinology* **56:** 299. 1955.
7. YUSHOK, W. D.: Ph.D. Thesis, Cornell University. 1950.
8. ROMANOFF, A. L.: *Bull. Alabama Polytech. Institute* **25:** 45. 1930.
9. HANAN, E. B.: *Am. J. Anat.* **38:** 423. 1927.
10. ROMANOFF, A. L.: *J. Exp. Zool.* **93:** 1. 1943.
11. HANSBOROGH, L. A. AND M. KHAN: *J. Exp. Zool.* **116:** 447. 1951.
12. STOLL, R. AND P. BLANQUET: *Ann. Endocrinologie* **14:** 1. 1953.
13. CARPENTER, E., J. BEATTIE AND R. D. CHAMBERS: *J. Exp. Zool.* **127:** 249. 1954.
14. MARAUD, R., R. STOLL, C. MACARIO AND P. BLANQUET: *Ann. Endocrinologie* **15:** 607. 1954.
15. ROMANOFF, A. L.: *Auk* **6:** 235. 1944.
16. ROMANOFF, A. L., L. L. SMITH AND R. A. SULLIVAN: *Cornell University Agr. Exp. Sta. Memoir* **216:** 1. 1938.
17. ANDREWS, F. N. AND E. E. SCHNETZLER: *Poultry Sci.* **25:** 124. 1946.

GROWTH HORMONE-INDUCED REDUCTION OF GLYCOSURIA AND PARTIAL REPAIR OF THE ISLETS OF LANGERHANS IN PARTIALLY PANCREATECTOMIZED DIABETIC RATS[1]

ADRIENNE A. BATTS,[2] LESLIE L. BENNETT, STANLEY ELLIS AND ROBERT A. GEORGE

The Department of Physiology and The Institute of Experimental Biology, University of California, Berkeley, California

SINCE it was demonstrated that administration of an extract of the anterior hypophysis results in permanent diabetes in several animal species, opinions have differed both as to which hypophyseal hormones affected the Islets of Langerhans and as to the mechanisms involved. With the advent of relatively pure preparations of the pituitary hormones, it has been shown that growth hormone (GH) is diabetogenic (1, 2, 3, 4) and is the only one of the highly purified hormones which will produce permanent diabetes in the intact animal (5). Although it has been pointed out that ACTH in small amounts will potentiate the effect of GH (6), ACTH alone in quite high dosage will not cause permanent diabetes. The permanent diabetes produced by GH is associated with anatomical abnormalities in the Islets of Langerhans and inability on the part of the pancreas to secrete insulin.

The mechanism by which GH produces these pancreatic abnormalities has received considerable attention. Evidence has been offered in support of the view that GH directly inhibits the secretion of insulin. Anderson and Long (7) report that GH added to the blood perfusing an isolated rat pancreas appears to inhibit the increased insulin production stimulated by hyperglycemia. It is reported that GH treatment produces a fall in the quantity of insulin extractable from the pancreas of the rat (8) and the dog (5, 9). On the other hand, a great deal of evidence supports the view that GH stimulates the islets to produce more insulin and that the permanent diabetes which develops is due probably to degeneration of the beta cells as a result of overwork. Repeatedly, anterior lobe extracts and purified GH preparations have been shown to cause lowering of the blood sugar (10, 11, 12, 13, 14, 15, 16), while Marks and Young (17) have found an increase in the insulin content of the rat pancreas after GH treatment. Also, Bornstein, Reid, and Young (18) have reported that the peripheral

Received May 28, 1956.

[1] This work was supported by a grant-in-aid (A-101) from the National Institute of Health.

[2] Holder of a post-doctoral fellowship from the National Foundation for Infantile Paralysis.

blood of a cat which has been treated for two days with GH will produce hypoglycemia in the alloxan-diabetic-hypophysectomized-adrenalectomized rat, an observation which they interpret as indicating an increased secretion of insulin by the pancreas of the cat. Histological studies have also indicated that the islets are stimulated when anterior lobe preparations are administered. The first claim for such an effect was advanced by Anselmino and coworkers (19, 20, 21) who observed an increase in the size and the number of islets, an increase in cell size, and a decrease in the blood sugar level following administration of a saline extract of acetone-dried pituitaries in several species, including the rat. Subsequently, several failures to repeat this work have been reported (22, 23), including a recent attempt with purified GH (24). On the other hand, other investigators (25, 26, 27, 28) have been able to produce similar changes using extracts prepared differently from those of Anselmino and coworkers. Richardson and Young (29) found that a saline extract of fresh pituitaries produced an increase in the islet mass, but the extract prepared by Anselmino's procedure using acetone-dried pituitaries was not effective. Also, Young (30), and Richardson and Young (31) have called attention to the fact that an unusual degree of mitotic activity is observed in the islets of dogs either during the early phase of GH treatment or during the time that the animals are becoming refractory to continued treatment. An increase in mitotic activity together with histological evidence of degeneration of the beta cells and a fall in insulin content of the pancreas in dogs receiving GH was observed by Best and coworkers (32) who also showed that treatment with insulin in addition to the anterior lobe extract prevented the beta cell degeneration and the fall in insulin content but did not prevent the increase in mitotic figures.

In many experiments carried out in this laboratory we have observed that the administration of GH to diabetic rats maintained on a constant food intake produces only a transient increase in glycosuria. This transient increase in glycosuria is followed by a decline in glycosuria to levels which usually are below those of the preinjection control period. This has been observed not only in partially pancreatectomized diabetic rats but also in alloxan diabetic rats. In an effort to throw further light on these observations and on the general question of the effects of GH upon the Islets of Langerhans we have carried out experiments in which the metabolic effects of administering GH to partially pancreatectomized diabetic rats was correlated with cytological changes occurring in the remaining Islets of Langerhans.

METHODS

Rats of the Long-Evans strain were partially pancreatectomized when they were about 30 days of age and were used in the experiments approximately 60 days after operation. That portion of the pancreas remaining between the common bile duct and the duodenum was left intact. Only animals with an established glycosuria were used

in the metabolic studies and they were maintained in individual, screen-bottomed metabolism cages over glass funnels containing porcelain disks to screen out feces. Urine was collected daily for the measurement of nitrogen and glucose. Urinary nitrogen was measured by the micro-Kjeldahl method and both blood and urinary glucose by the method of Nelson (33). A constant, somewhat restricted quantity of food was provided daily, with the result that all food given was eaten. This ensured a constant food intake throughout the metabolic period.

In all experiments a minimum of five animals was used in each group. One group of animals received 5 international units per rat per day of commercial ACTH (Armour's). When GH was given the dose was 5 mg. per day per rat, given intraperitoneally. The animals were sacrificed together with 4 partially pancreatectomized, non-diabetic controls and 4 normal controls. At autopsy, the pancreas remaining between the common bile duct and the duodenum was removed and fixed in Bouin's for the Gomori chrome hematoxylin-phloxine stain (34) and for the iron-hematoxylin-analine blue (IHAB) stain of Koneff (35).

In order to estimate cell size, tracings were made of large numbers of cells from different islets by means of a camera lucida. The total area occupied by the cells traced for each animal was measured in arbitrary units by means of a planimeter and the figure obtained was divided by the number of cells traced. This gives a numerical value which represents in these arbitrary units the average cell size for that animal and which allows comparison of islet cell sizes between groups of animals.

RESULTS AND COMMENTS

Metabolic Observation

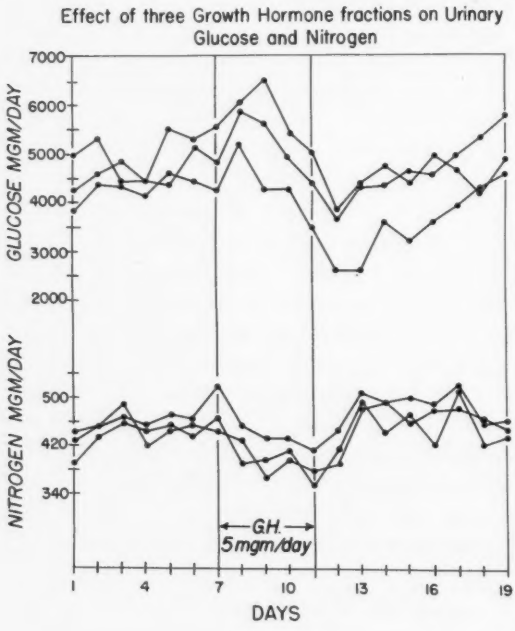


FIG. 1

In Figure 1 are shown the average glycosuria and urinary nitrogen of three groups of partially pancreatectomized, diabetic rats that were given GH for 4 days. It is apparent that the pattern of response in all groups is similar. The urinary nitrogen excretion declines in all groups during the period of GH injection and remains low as long as the hormone is given. As soon as hormone administration is stopped, nitrogen excretion rises promptly to the preinjection levels. The glycosuria increases for the first and sometimes the second day of GH injection. It then decreases with continued GH administration and continues to fall for one or two days after cessation of GH treatment. This is followed by a gradual

return to the preinjection control level. In the course of one experiment in which GH was administered for 6 weeks, the urine glucose declined for the first 10 days of treatment and then stabilized even in the face of as much as 10 mg. of hormone per day. After cessation of treatment the glycosuria increased again to the preinjection control level.

In Figure 2 are shown the average glycosuria and urinary nitrogen of the three groups of rats whose pancreatic remnants were studied histologically at the end of the injection period. It will be observed that no particular change in either glycosuria or urinary nitrogen occurred in the uninjected control group. The animals which received GH exhibited a decline in urinary nitrogen which persisted throughout the treatment period. These same animals showed an initial increase in glycosuria followed by a decline in glycosuria so that on the 6th day of treatment the level of glycosuria was appreciably below its preinjection control level. In contrast, the rats which received ACTH exhibited an increase in both glycosuria and urinary nitrogen which was maintained throughout the treatment period.

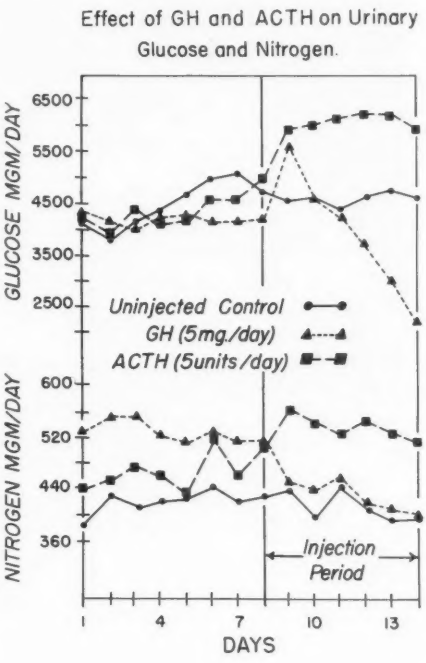


FIG. 2

Hisiological Observations

Normal control: The Gomori stain revealed islets consisting largely of beta cells which contained abundant blue-staining granules. The alpha cells also contained numerous granules which stained with phloxine.

Although the IHAB stain is not a differential islet stain, in tissues stained by this technique, it was possible to distinguish alpha cells from beta cells, since the alpha cells were smaller, and more darkly stained. The beta cells were relatively uniform in size, with sharp cell borders and a somewhat granular cytoplasm. The nuclei were all generally of the same size. Only a few strands of connective tissue were visible among the cells (Fig. 3).

Diabetic, untreated: In all cases, the islets showed marked damage, the changes being more pronounced in some animals than others. In sections stained by the Gomori technique the beta cells were almost completely de-

3

4

granulated and only occasional alpha cells were to be found. Because of the loss of specific granulation and because of the superiority of IHAB as a cytological stain, the descriptions which follow are based upon study of sections stained with IHAB. The islet cells were greatly enlarged and showed indistinct borders. The cytoplasm appeared clumped and often showed marked vacuolation (Fig. 4). In most cells, the nuclei were enlarged and vesicular and contained very prominent nucleoli, but some pyknotic nuclei were visible. In fact, it was possible to distinguish cells in all stages of deterioration, with the apparent end stage being an empty hole in the islet bridged by a few strands of cytoplasm with a densely pyknotic nucleus lodged on one side. In all cases, the amount of fibrous tissue in the islets was greatly increased, so that in extreme examples it would have been scarcely possible to ascertain where the islet had been except for the presence of a few scattered, much damaged cells (Fig. 5). In some animals there appeared to be formation of new islets, as judged by the complete absence of fibrous tissue, but even these showed marked degenerative changes in the cells.

Measurements of cell size in this group revealed a marked degree of enlargement of the islet cells as compared with those of the normal controls (Table 1).

Diabetic, treated with GH: At the end of 6 days of GH treatment, tissues stained by the Gomori method still showed an almost complete absence of granulation in the beta cells and also an almost complete absence of alpha cells. The IHAB stain in all animals showed to some degree what seemed to be a considerable improvement in the condition of their islet cells (Figs. 6 and 7). In most cases, while the cells were still very large, the cell borders were quite sharp and the cytoplasm relatively homogeneous. No new degeneration of cells appeared to be taking place; in fact, all spaces in the islets apparently left by cells which had previously degenerated were lined with connective tissue fibers by the time of autopsy. As in the untreated diabetics, new islets were apparently being formed, but in the GH treated animals these islets appeared almost normal except for the large size of the cells.

After 2 days of GH treatment, there was a suggestion of improvement in the condition of the islets, but the changes were not as marked as after 6 days of treatment. No early structural changes were found which could be interpreted as bearing any causal relation to the initial increase in glycosuria.

The average cell sizes in the groups of animals treated with GH for both

---

FIG. 3. Normal control pancreas, showing a single islet. ×485. IHAB stain.

FIG. 4. Untreated diabetic islet. ×485 IHAB stain. Note large size of cells and nuclei, indistinct cell borders, numerous vacuolated cells and extensive invasion of connective tissue. No acinar tissue shown.

5

6

TABLE 1. CELL SIZE IN THE ISLET OF NORMAL AND DIABETIC RATS

Experiment		Average cell size in arbitrary units
I	Normal control	265* (209, 225, 306, 321)†
	Untreated diabetic	420 (378, 408, 408, 420, 485)
	Diabetic plus GH 2 days treatment	442 (324, 404, 422, 423, 477, 482, 495, 508)
	Diabetic plus GH 6 days treatment	460 (392, 419, 420, 463, 491, 514, 521)
Experiment II	Normal Control	234 (195, 229, 256, 256)
	Untreated diabetic	452 (409, 409, 457, 489, 494)
	Diabetic plus ACTH 2 days treatment	404 (335, 419, 458)
	Diabetic plus ACTH 6 days treatment	319 (289, 293, 333, 360)

* Average for entire group.
† Cell size in individual animals.

2 and 6 days did not differ significantly from the average values observed in the untreated diabetic group (Table 1).

Presumably the beta cells in the pancreatic remnants of the diabetic rats are secreting insulin at a maximum rate and one can assume that the observed damage is the result of overwork. The finding that GH treatment simultaneously lowers the urine sugar and improves the appearance of the beta cells implies that GH has in some way increased the functional capacity of the beta cells. Certainly the improved appearance of the Islets of Langerhans correlates well with the reduction of glycosuria. Kurtz and collaborators (14) have reported that GH produces hypoglycemia even in the absence of the pancreas. It might be argued that GH by some extrapancreatic mechanism reduces hyperglycemia and glycosuria and that the changes in the islets are a secondary rather than a primary effect of the GH. The observation, in preliminary experiments, that the adminstration of small doses of insulin (sufficient to reduce glycosuria comparable to that reduction produced by GH) does not alter the islets is against this interpretation. Also against this interpretation is the observation of Milman and Russell (36) that GH has only a hyperglycemic effect in the rat with alloxan diabetes.

FIG. 5. Untreated diabetic ×485. IHAB stain. Note almost complete disappearance of islet cells with replacement by connective tissue.

FIG. 6. Diabetic treated with GH for 6 days. ×485. IHAB. Note scarcity of degenerative changes seen in Figures 4 and 5. Cell borders are very distinct, cytoplasm homogeneous, and connective tissue at a minimum. No acinar tissue is shown.

7

8

Diabetic, treated with ACTH: The animals treated with ACTH showed islets which either were indistinguishable from those of untreated diabetics or gave evidence of even more severe damage. In addition to the changes in the islets of the diabetic rats already described, the ACTH-treated animals showed an unusual number of hemorrhages into the islets as well as some increase in the degree of clumping of the cytoplasm and some additional loss of distinctness of the cell borders (Fig. 8).

In Table 1, the figures for average cell size in the groups treated with ACTH are somewhat smaller than those for the untreated diabetics. Because of the extreme indistinctness of the cell borders in this group, it was very difficult to be sure that the cells were being correctly traced, so that the significance of these figures is doubtful.

The above histological observations are of particular interest since they correlate with the increase in glycosuria which was maintained by these animals throughout the treatment period. Also they tend to strengthen the view that the GH effect is a specific one.

SUMMARY

The administration of GH to diabetic rats initially increases their glycosuria; however, in spite of continued treatment, the glycosuria decreases below preinjection levels. Since this could depend upon changes in activity of the islets, a metabolic-histologic experiment was performed. Partially pancreatectomized diabetic rats were sacrificed before and after GH treatment and the cytological findings were correlated with the diabetic status.

In all untreated diabetic animals, the islets showed enlarged cells and almost complete disappearance of specific granulation together with extensive vacuolation and other degenerative changes. Islets showed varying degrees of fibrosis, the extreme being a fibrotic islet containing a few scattered deteriorating cells. In no case was it possible to distinguish alpha cells. In the animals treated with GH for 6 days, there was remarkable improvement in the appearance of the islets, although it was still impossible to demonstrate specific granulation of alpha and beta cells. The cells were numerous, large, and had sharp borders, and were filled with homogeneous cytoplasm showing little evidence of injury. New areas of healthy cells were to be found in old fibrotic islets. On the other hand, administration of ACTH for 6 days caused some apparent reduction of cell size, together with reduction in clarity of cell outlines and evidence of augmented cellular damage. Hemorrhages into the islets were frequent in these animals.

FIG. 7. Diabetic treated with GH for 6 days. ×485. IHAB. Note cluster of possible newly regenerated cells which appear relatively normal.

FIG. 8. Diabetic treated with ACTH for 6 days. ×485. IHAB stain. Note pyknotic nuclei and very indistinct cell borders.

The changes in the islets of diabetic animals are interpreted as being due to overwork of the cells of the pancreatic remnant. The finding of healthy new cells following GH administration is in accord with the decrease in urine sugar observed and suggests that the GH in some way permits the beta cells to hypertrophy more effectively in response to the stimulus of an elevated blood sugar.

REFERENCES

1. Houssay, B. A. and R. R. Rodriguez: *Endocrinology* **53**: 114. 1953.
2. Cotes, P. M., E. Reid and F. G. Young: *Nature* **164**: 209. 1949.
3. Campbell, J., I. W. F. Davidson, W. D. Snair and H. P. Lei: *Endocrinology* **46**: 273. 1950.
4. Campbell, J., H. R. Hausler, J. S. Monroe and I. W. F. Davidson. *Endocrinology* **53**: 134. 1953.
5. Campbell, J., L. Chaikoff and I. W. F. Davidson: *Endocrinology* **54**: 48. 1954.
6. Young, F. G.: *Rec. Prog. Horm. Res.* **8**: 471. 1953.
7. Anderson, E. and J. A. Long: *Endocrinology* **40**: 98. 1947.
8. Fraenkel-Conrat, H., V. V. Herring, M. E. Simpson and H. M. Evans: *Am. J. Physiol.* **135**: 404. 1942.
9. Best, C. H., J. Campbell and R. E. Haist: *J. Physiol.* **97**: 200. 1939.
10. Scott, J. L., Jr. and F. L. Engel: *Endocrinology* **46**: 582. 1950.
11. Marx, W., V. V. Herring and H. M. Evans: *Am. J. Physiol.* **141**: 88. 1944.
12. Weinstein, R. C.: *Proc. Soc. Exper. Biol. and Med.* **40**: 667. 1939.
13. Shpiner, L. B. and S. Soskin: *Am. J. Physiol.* **109**: 97. 1934.
14. Kurtz, M. R. C. deBodo, S. P. Kiang and A. Ancowitz: *Proc. Soc. Exper. Biol. and Med.* **76**: 21. 1951.
15. Conn, J. W. and L. Louis: *J. Clin. Endocrinol.* **5**: 247. 1945.
16. Zunz, E. and J. leBarre: *Proc. Internat. Physiol. Cong.* pg. 135. 1935.
17. Marks, H. P. and F. G. Young: *Lancet* **1**: 493. 1940.
18. Bornstein, J., E. Reid and F. G. Young: *Nature* **168**: 903. 1951.
19. Anselmino, F. G., L. Herold and F. Hoffman. *Ztschr. f. d. ges. exper. Med.* **97**: 329. 1935.
20. Anselmino, F. G., L. Herold and F. Hoffman: *Klin. Wchnschr.* **12**: 1245. 1933.
21. Anselmino, F. G. and F. Hoffman: *Klin. Wchnschr.* **12**: 1435. 1933.
22. Leyton, O.: *Proc. Roy. Soc. Med.* **29**: 723. 1936.
23. Elmer, A. W., B. Giedosz and M. Scheps: *Compt. Rend. Soc. de Biol.* **124**: 823. 1937.
24. Abrams, G. B., B. L. Baker, D. J. Ingle and C. H. Li: *Endocrinology* **53**: 252. 1953.
25. Chrzanowski, B. and S. J. Grzycki: *Klin. Wchnschr.* **16**: 488. 1937.
26. Haist, R. E. and B. Kinash: *Fed. Proc.* **10**: 58. 1951.
27. Ogilvie, R. F.: *J. Path. & Bact.* **56**: 225. 1944.
28. Mount, L. E.: *J. Physiol.* **114**: 1. 1951.
29. Richardson, K. C. and F. G. Young: *J. Physiol.* **91**: 352. 1937.
30. Young, F. G.: *Biochem. J.* **32**: 513. 1938.
31. Richardson, K. C. and F. G. Young: *Lancet* **1**: 1098. 1938.
32. Best, C. H., J. Campbell, R. E. Haist and A. W. Ham: *J. Physiol.* **101**: 17. 1942.
33. Nelson, N.: *J. Biol. Chem.* **153**: 375. 1944.
34. Gomori, G.: *Am. J. Path.* **17**: 395. 1941.
35. Koneff, A. A.: *Anat. Rec.* **66**: 173. 1936.
36. Milman, A. E. and J. A. Russell. *Endocrinology* **47**: 114. 1950.

DENSITY AS AN INDEX OF THE EFFECTS OF ESTROGENS ON BONE

RICHARD A. EDGREN AND DAVID W. CALHOUN
Division of Biological Research, G. D. Searle and Co., Chicago, Illinois

THE discovery that the proliferation of medullary bone paralleled the follicular phase of the ovarian cycle in pigeons by Kyes and Potter (1) led to extensive studies on the effects of estrogenic materials in various birds and mammals. Zondek (2) using domestic fowl, Pfeiffer and Gardner (3) using English Sparrows and Gardner and Pfeiffer (4) using mice showed that exogenous estrogens cause bony proliferation in these species. In most such studies growth has been determined from sections of the long bones. Studies, too numerous to cite, employing mice, rats and birds of various species have made direct measurements of organic and mineral content of bones from estrogen treated animals. These have generally shown relative increases of mineral and decreases of organic components under estrogen treatment. Recently (5) radioactive tracer techniques have been used.

The need for a quantitative technique for comparing the effects of various estrogens on bone has been obvious for some time. Urist, Budy and McLean (6) partially filled this need by combining histological and roentgen studies during and after estrogen-treatment of mice. Responses could be scored on a 0 to +4 basis, the lower levels of activity requiring histological verification. These studies showed increased deposition of endosteal bone with increased amounts of estradiol benzoate (β-isomer). Their tables suggested that estradiol benzoate gave a linear log dose-response curve over a period of three weeks (total doses of 1, 2 and 4 mg.). Through a 5-week period, diethylstilbestrol at the same doses gave a similar curve, but at a lower level of response.

In this laboratory we have studied bone density as a measure of the effects of estrogens in increasing bony proliferation. In a previous note (7) preliminary experiments with English Sparrows were described. The present communication summarizes studies employing mice as the experimental animals.

MATERIALS AND METHODS

The left femur has been used to obtain an estimate of bone density. All animals were sacrificed under chloroform anaesthesia and at autopsy the left femur was removed grossly. After removal the femora were autoclaved at 15 pounds pressure for 15 minutes and cleaned by hand of all adherent tissues. Preliminary experiments showed that it was virtually impossible to clean the bones adequately without accidentally removing

Received June 15, 1956.

some of the epiphyses; in subsequent experiments (all those reported here) the epiphyses were removed routinely.

After cleaning, the bones were weighed twice to the nearest 0.05 mg. on a 0–100 mg. Roller-Smith torsion balance. The bones were first weighed suspended in distilled water and, second, after careful blotting, suspended in air. To reduce the effects of surface tension on the suspending fibre, 20 mg. of a wetting agent (Wetanol) were added to each 100 ml. of water. Water temperature was measured to the nearest degree centigrade. Femoral densities were calculated from the following relationship:

$$\rho_x = \frac{\rho_1}{1 - \frac{W_1}{W_a}}$$

where ρ_x is the density of the bone; ρ_1 the density of water at the temperature at which the weighing was made; W_1 the bone weight suspended in water; and W_a the bone weight in air.

Statistical computations have been based on Bliss and Calhoun (8).

Experiment 1. Preliminary studies had indicated that diethylstilbestrol (stilbestrol) at various dose levels caused significant increase in bone density when administered over approximately three weeks. In order to ascertain the most satisfactory duration of treatment, a series of 100 20–30 gram, male, white mice were divided into 10 groups of 10 mice each. Groups 1 and 2 were sacrificed immediately. Of the remainder, the odd numbered groups were treated daily with 40 μg. of stilbestrol in 0.05 ml. corn oil while the even numbered groups received similar quantities of oil. Of these, one stilbestrol-treated and one control group were sacrificed each week for the next four weeks.

Experiment 2. As the results of experiment 1 suggested that three weeks of treatment produced a satisfactory differentiation between treated and control groups, this period was chosen for assay purposes. Over a period of several months, tests employing stilbestrol, estrone and estradiol-17β were carried out. The 21 daily injections were contained, as above, in 0.05 ml. of corn oil, and the animals were sacrificed on the day after the final injection. Most studies employed intact adult (25–30 g.) male white mice, although a few utilized adult females, and a few more, males castrated a week before injections were begun.

RESULTS

Experiment 1. The daily administration of 40 μg. of stilbestrol caused a significant increase in density of the femur (Table 1). The oil-treated controls also showed significant density increases over the first two or three weeks and then began to level off to a density of approximately 1.53. In

TABLE 1. INCREASE OF FEMORAL DENSITY IN MICE RECEIVING 40 μG. OF STILBESTROL DAILY

Days of treatment	Controls	Treats	Difference ± S.E.†
0	1.4536	—	
7	1.4659	1.4927	0.0268 ± 0.0167*
14	1.5090	1.5782	0.0692 ± 0.0166**
21	1.5354	1.6248	0.0894 ± 0.0172***
28	1.5310	1.6662	0.1352 ± 0.0176***

* $P < 0.05$ that treat men = control mean.
** $P < 0.01$ that treat mean = control mean.
*** $P < 0.001$ that treat mean = control mean.
† S.E. $= \sqrt{(\text{s.e.}_1)^2 + (\text{s.e.}_2)^2}$

TABLE 2. ESTIMATES OF VARIABILITY IN FEMORAL DENSITY OF OIL-INJECTED CONTROL MICE

Statistic	Estimated value	99% confidence
Long Term Average (μ)	1.5352	1.5192 to 1.5512
Standard Deviation (σ)	0.0470	Less than 0.0580
For One Group (10 animals)		
Mean ($\bar{x}$)	1.5352	1.4768 to 1.5938
Standard Error ($s_{\bar{x}}$)	$\pm$0.0148	Less than $\pm$0.0250

the stilbestrol-treated mice the increase was faster than in the controls so that the difference between groups and level of significance became progressively greater with increased time. The three-week injection period seemed to promise treat-control differentiation at a comfortable level of significance and so was chosen for comparative studies.

Experiment 2. Control Groups. The mean density of 131 oil-injected control mice was 1.5352 (15 groups, usually with 10 mice per group). The variability was not the same in all of these groups: in nine the variance (s^2) was between 0.0015 and 0.0038, while in the remainder s^2 was less than 0.0008. Taking the more variable groups as the basis of estimation, the average standard deviation of control densities appeared to be 0.0470 and almost certainly (99% confidence) did not exceed 0.0580. These estimates are probably larger than the true figures as a result of omitting the low variability groups. The findings are summarized in Table 2. Data on other control animals of various types are presented in Table 3. All of these values fall within the ranges expected from the data of Table 2, indicating that neither sex, nor castration, nor solvent significantly affected bone density.

Treatment Groups. The three standard estrogens (stilbestrol, estradiol and estrone) produced linear regression of femur density on the log of the daily dose within the dosage range used (Fig. 1). In all cases as the daily dose was increased the density was increased in proportion to the log of the dose. The bone-density response appeared to be homoscedastic; that is the variability did not change significantly over the range of the dose-response

TABLE 3. FEMUR DENSITIES OF VARIOUS TYPES OF CONTROL MICE

Condition	N	Mean $\pm$S.E.
Intact males		
Sesame oil	10	1.5037 $\pm$0.0130
Saline	10	1.5159 $\pm$0.0116
Untreated	10	1.5302 $\pm$0.0075
	5	1.5486 $\pm$0.0054
	5	1.5290 $\pm$0.0193
Intact females	10	1.5453 $\pm$0.0100
Corn oil	10	1.5549 $\pm$0.0103
	9	1.5636 $\pm$0.0097
Castrate males		
Corn oil	9	1.5527 $\pm$0.0213

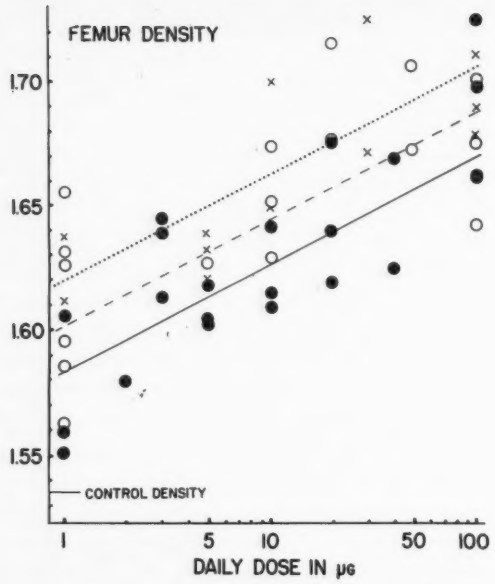


FIG. 1. Regression of femur density on log dose of diethylstilbestrol (solid line and dots); estradiol (dashed line and circles); and estrone (dotted line and crosses).

curve. Gross inspection of the curves suggesting parallelism among the three lines was confirmed by an F-test which showed a single, pooled slope to be satisfactory ($F = 1.09$). The formulae for the curves are presented in Table 4, together with potency estimates on the three compounds.

The several experiments employing castrated males and intact adult females showed that responses under these conditions did not differ greatly from those obtained with intact adult males. Neither sex nor castration grossly affected the bone response to estrogens (Table 5).

DISCUSSION

The results of the stilbestrol groups in experiment 1 suggested the three-week period as most efficient for comparative purposes. The increased density in the oil-treated groups probably reflected an age change, as similar changes have been reported in untreated animals (9). Landauer

TABLE 4. REGRESSION FORMULAE OF FEMUR DENSITY (y) ON LOG OF DAILY DOSE OF ESTROGEN (x). N, NUMBER OF MICE USED IN CALCULATIONS

Estrogen	N	Formula	Rel. potencies
Stilbestrol	174	$y = 1.5834 + 0.0423x$	1.00
Estradiol	125	$y = 1.6019 + 0.0423x$	2.74
Estrone	58	$y = 1.6200 + 0.0423x$	7.33

$b = 0.0423 \pm 0.0057$

TABLE 5. EFFECTS OF STILBESTROL AND ESTRADIOL ON BONE DENSITY OF INTACT ADULT FEMALE AND CASTRATED MALE MICE COMPARED WITH EXPECTATIONS FROM DATA ON INTACT ADULT MALES

	N	Observed	Expected range*
Castrate Males			
20 μg.	10	1.6618	1.6084–1.6684
Females			
Stilbestrol			
5 μg.	10	1.6181	1.5834–1.6426
10 μg.	9	1.6551	1.5945–1.6569
40 μg.	2	1.6780	1.5846–1.7178
100 μg.	7	1.6363	1.6304–1.7056
Estradiol			
1 μg.	9	1.6550	1.5688–1.6352
1 μg.	9	1.6310	1.5688–1.6352
10 μg.	8	1.6564	1.6112–1.6772
20 μg.	8	1.6773	1.6238–1.6902
100 μg.	3	1.6753	1.6313–1.7417

* "Expected Range" equals expected value (from equations, table 4) ± twice the standard error of the difference between observed and expected values. About 95% of observed means would be expected to fall within stated range.

(10) has shown that corn oil contains a material with an action similar to vitamin D. Such an explanation would seem inapplicable here in view of the density similarities of oil-treated controls to untreated groups maintained through the treatment period (Table 3). Also the 3- and 4-week oil-controls had leveled off at mean values similar to those obtained for the 131 controls of experiment 2. Attempts to correct statistically for the body weight regression of bone density have proved unsuccessful because the differing catabolic potencies of the estrogens employed introduce complications (unpublished data). We have attempted a correction technique employing the weight of the bone suspended in air as a second measured variable; however, the labor of the correction was not warranted by the reduction of variability obtained. Further, the narrow weight range of animals employed tended to obviate this difficulty.

These studies suggested that, over the range of dose levels employed, estrone was the most potent estrogen in causing proliferation of medullary bone, being roughly three times as potent as estradiol and seven times as potent as diethylstilbestrol. These suggested potency relationships are not in complete agreement with the results obtained by Urist, Budy and McLean (6), who found that these substances were of approximately equal potency in some experiments, and that estradiol was somewhat more potent in others.

SUMMARY

Estrogenic substances stimulated increases in the densities of femora of treated mice, presumably by causing proliferation of medullary bone. Administration of a constant daily dose of diethylstilbestrol increased density with increased time of administration. Diethylstilbestrol, estradiol and

estrone all showed parallel log dose-response lines. Estrone appeared to be the most potent and diethylstilbestrol the least.

Acknowledgments

Thanks are extended to Mr. H. S. Backus of this company for assistance with the physical aspects of this problem, and to Miss Nancy Hanway and Mrs. Grace Perkins for technical aid.

REFERENCES

1. Kyes, P. and T. S. Potter: *Anat. Rec.* **60**: 377–379. 1934.
2. Zondek, B.: *Lancet*: 842. 10 Oct., 1936.
3. Pfeiffer, C. A. and W. U. Gardner: *Endocrinology* **23**: 485. 1938.
4. Gardner, W. U. and C. A. Pfeiffer: *Proc. Soc. Exp. Biol. & Med.* **37**: 678. 1938.
5. Govaerts, J. M., M. J. Dellemagne and J. Melon: *Endocrinology* **48**: 443. 1951.
6. Urist, M. R., Ann M. Budy and F. C. McLean: *J. Bone & Joint Surg.*, **32-A**: 143. 1950.
7. Edgren, R. A.: *Endocrinology*, **56**(4): 491. 1955.
8. Bliss, C. I. and D. W. Calhoun: *An outline of biometry*, Yale Co-op. Corp., New Haven: 1954.
9. Edgren, R. A.: In preparation. 1956.
10. Landauer, W.: *Endocrinology* **55**: 686. 1954.

DIABETOGENIC ACTION OF PITUITARY HORMONES ON ADRENALECTOMIZED HYPOPHYSECTOMIZED DOGS

B. A. HOUSSAY AND J. C. PENHOS

Institute of Biology and Experimental Medicine Costa Rica 4185-Buenos Aires, Argentina

THE diabetogenic action of the anterior pituitary extracts was demonstrated in hypophysectomized-pancreatectomized toads by Houssay and Biasotti (1). This action was not obtained in pancreatectomized cats and rats in the absence of adrenals (2, 3). But the diabetogenic action of the anterior pituitary extract was obtained in other adrenalectomized animals: a) toads deprived of adrenals and pancreas (4); b) toads deprived of hypophysis, pancreas, kidney and adrenals (5): c) toads deprived of hypophysis, pancreas and adrenals (4, 6); in cases *b* and *c*, the diabetogenic action was diminished by the absence of the adrenals. The diabetogenic action of the pituitary was also observed in mammals deprived of the adrenals such as: d) dogs with reduction of the pancreatic mass, deprived of both adrenals and maintained with cortico-adrenal extract (7, 8) or with desoxycorticosterone (8); e) dogs with reduction of the pancreatic mass, deprived of the adrenals, maintained only with sodium chloride and not receiving adrenal hormones (8); some of these dogs were also deprived of the thyroid. All these experiments demonstrated that hypophyseal diabetes may be obtained without the presence or participation of the adrenals. In dogs deprived of pancreas and adrenals, somatotrophin administration increased the resistance to insulin and produced glucose tolerance curves of diabetic types (9, 10). Somatotrophin also increase the resistance to insulin in adrenalectomized dogs (11).

Prolactin had a diabetogenic effect: a) in hypophysectomized-pancreatectomized toads (12, 13); b) in dogs (13, 14, 15); and cats (13, 14) with large reduction of the pancreatic mass. This hormone had also a certain antiinsulinic effect in dogs deprived of hypophysis and adrenals (9, 10, 16).

The present paper reports that the diabetogenic effect of somatotrophin, prolactin, adrenocorticotrophin and hydrocortisone was found in dogs with pancreas reduced to 15–18% of its mass, deprived of hypophysis and adrenals and maintained only with sodium chloride.

PLAN AND METHODS

Experiments were carried out in dogs deprived in the first operation of 82 to 85% of the pancreatic mass, in the second operation of the right adrenal, in the third of the hypophysis and, finally, in the fourth of the left adrenal.

Received June 16, 1956.

They received at different times subcutaneous injections of growth hormone (Wilson, lot 95567), prolactin (Bates, sample 71713) and adrenocorticotrophin (Armour, 146RS3, potency 3.65 the standard) at the doses indicated in the table, in 1 ml. of 0.8% Na Cl solution, during 4 to 5 days. Glycemia was determined daily according to the Somogyi-Nelson technique collecting blood from cuts on the ear edge, after 16 hours of fasting. The diabetic response was considered positive when the fasting blood glucose level, after the injection rose from the normal level to 150 mg. per 100 ml. of blood. The next assay was carried out at least 8 days after glycemia had returned to normal. These dogs were also injected subcutaneously with cortisol.

The animals were fed fresh or cooked meat (250 to 300 g./day). After hypophysectomy and after the second adrenalectomy they received: the first three days, a daily dose of 1 mg. of desoxycorticosterone acetate. Cortisone acetate was also injected: 2 mg. twice a day during two days, 1 mg. twice a day during the following three days and then it was discontinued. After the second ardenalectomy, they were given the first days 200 ml. of saline solution twice a day and later on, the only treatment was sodium chloride (4 to 5 g./day) given mixed with boiled beef meat. These animals required a constant care in order to maintain them in good conditions.

When the animals became asthenic or depressed (most of the time with hypoglycemia) or when they lay on the floor, walking only with great difficulty, they were given 0.5 mg./kg. of cortisone acetate, during 2 days. With this treatment they recovered strength and the blood sugar level (48–60 mg./100 ml.) returned to normal.

RESULTS

The dates on which the 3 dogs were operated as well as the time of study are represented in Table 1. Dog 66 was a female and Dogs 77 and 78 were males.

General symptoms.—The animals with pancreatic mass reduced and deprived of the right adrenal, gained weight rapidly after hypophysectomy and showed a big appetite; in two months, the body weight increased in Dog 66 from 6 kg. to 9.5 kg. (+3.5); Dog 77 from 6 to 7.2 kg. (+1.2) and Dog 78 from 8.9 to 10.3 (+1.4); adiposity was remarkable.

In general, they walked and moved slowly, specially Dog 66. Dogs 77 and 78 barked in the presence of persons unknown to them or when seeing cats. Dog 66 was the most excited in the last case. Weakness of the posterior legs was often observed and sometimes Dog 66 was unable to climb a staircase. She was extremely tame and docile.

Table 2 shows the initial glycemia and the highest level reached during the 4 to 5 days of treatment. It can be observed that somatotrophin (1 mg./kg./day) produced a diabetic response in the 3 dogs deprived of hypophysis, adrenals and a large part of the pancreas.

Prolactin administration of 1 mg./kg./day, also produced a diabetic response in Dogs 66 and 77 and in doses of 2–10 mg./kg./day in dog 78. Adrenocorticotrophin had no diabetogenic effect in these adrenalectomized dogs at doses which were active in Dog 66 and others before being deprived of the adrenals.

Hydrocortisone was active in these dogs deprived of the hypophysis and the adrenals and with large partial pancreatectomy, provoking a

TABLE 1. DATES OF OPERATION, AMOUNT OF PANCREAS REMOVED AND DURATION OF OBSERVATION

No. of dog	Pancreas removed %	Pancreatectomy	Removal of right adrenal	Hypophysectomy	Removal of left adrenal	Duration of observation, days
66	82	29 April	18 Dec.	28 Nov.	7 March	375
77	85	13 Oct.	7 Dec.	15 Dec.	22 Feb.	180
78	85	13 Oct.	6 Dec.	14 Dec.	29 March	201

rise in the blood sugar level from 110 to 190 mg./100 ml. in Dog 66; from 98 to 185 mg./100 ml. in Dog 77 and from 76 to 170 mg./100 ml. in Dog 78.

The effect of 5 mg. of Prolactin was reinforced by the addition of 2 mg. of adrenocorticotrophin given to Dog 66 before removing hypophysis and adrenals, as glycemia rose from 89 to 209 mg./100 ml. No change was observed with the separate administration of these substances.

Although the diabetes was, in most cases, of the temporary type, there were cases of 9 and 11 days duration, after 4 days of treatment in Dog 66, before and after removing the adrenals and the hypophysis. Dog 77 showed permanent diabetes after receiving 2 mg./kg./day of hydrocortisone during

TABLE 2. DIABETOGENIC ACTION OF PITUITARY HORMONES IN DOGS WITH PARTIAL PANCREATECTOMY (PART. PANC.), HYPOPHYSECTOMIZED (HYP.), DEPRIVED OF THE RIGHT ADRENAL (UNIL. ADR.) OR BOTH ADRENALS (BIL. ADR.). INITIAL AND MAXIMAL GLYCEMIA DUE TO DAILY ADMINISTRATION OF HORMONES DURING 4 TO 5 DAYS

Dog	Somatotrophin		Prolactin		Adrenocorticotrophin	
	Mg./kg./day	Glycemia	Mg./kg./day	Glycemia	Mg./kg./day	Glycemia
Female No. 66						
Part. panc., 82%	1	100–257	2	84–125	2	82–102
	1	75–211	5	95–107	3	80–152
			10	90–153		
Part. panc., Hyp. Unil. adr.	1	76–116	—	—	—	—
	2	95–335	—	—	—	—
Part. panc., Hyp., Bil. adr.	1	86–200	1	92–268	3	92–75
			5	104–195	—	—
Male No. 77						
Part. panc.	1	94–104	—	—	—	—
	2	95–165	—	—	—	—
Part. panc., Hyp., Bil. adr.	1	102–248	1	92–166	3	83–88
Male No. 78						
Part. panc.	1	91–203	—	—	—	—
Part. panc., Hyp., Bil. adr.	1	92–206	1	84–115	3	67–79
	—	—	2	89–250	—	—
	—	—	10	81–260	—	—

4 days. In the other cases, glycemia returned to normal levels one to three days after discontinuing the treatments.

DISCUSSION

Adrenalectomized-hypophysectomized dogs can survive a long time when fed meat and sodium chloride.

It was demonstrated once more, that the adrenals and their hormones are not essential for the production of the diabetogenic effect of somatotrophin and prolactin. Thus diabetes was obtained in dogs deprived of adrenals and hypophysis, with pancreas previously reduced to 15–18% of its mass. This diminution of the pancreatic mass made the animals markedly sensitive to the mentioned hormones, thus allowing diabetes to develop using lower and nontoxic doses.

Former investigations on pancreatectomized-hypophysectomized toads, demonstrated that the diabetogenic action of the hypophysis was less intense in those animals deprived of the adrenals. Reciprocally the diabetogenic action of somatotrophin was increased in the dog by corticoid administration (17).

Somatotrophin was more active than prolactin, as in the previous experiments. The prolactin used did not contain somatotrophin (no increase of body weight after injection in hypophysectomized rats, 10 mg. in 10 days). The action of somatotrophin and prolactin may be an intrinsic property of the hormones themselves or of any component or impurity not essential for their specific effect. Their action is not due to adrenocorticotrophin as these hormones had diabetogenic cation and adrenocorticotrophin had no diabetogenic action in the dogs studied deprived of adrenals and hypophysis.

The marked adiposity observed in the dogs deprived of a large part of the pancreas, hypophysis and one adrenal was associated with hyperphagia. The possible metabolic changes were not studied. This adiposity decreased during the periods of hyperglycemia or diabetes.

Quietness and a tendency to sit or lie on the floor was evident in these animals. The slow movements of the posterior legs seem to be due to asthenia or muscular weakness, but no signs of articular alterations were observed.

These dogs maintained their normal behavior such as barking in the presence of unknown people or cats.

CONCLUSION

The pituitary hormones, somatotrophin and prolactin, had a diabetogenic effect in dogs deprived of hypophysis and adrenals and with pancreas surgically reduced to 15–18% of its mass. These facts demonstrate that the hypophysis and the adrenals are not essential for the production of the diabetogenic effect of these hormones.

Adrenocorticotrophin had no action on the glycemia of these adrenalectomized dogs.

Some of the animals showed moderate symptoms of asthenia in others these were marked. The animals recovered rapidly when injected with cortisone or hydrocortisone.

Hydrocortisone, at a dose of 3 mg./kg./day, produced in a short time, a diabetic hyperglycemia.

The dogs with reduced pancreas and deprived of one adrenal and the hypophysis developed a marked adiposity.

REFERENCES

1. Houssay, B. A. and A. Biasotti: *Rev. Soc. argent. Biol.* **6**: 8. 1930; *C. R. Soc. Biol.*, Paris., **104**: 407. 1930.
2. Long, C. N. H. and F. D. W. Lukens: *J. exp. Med.* **63**: 465. 1936.
3. Long, C. N. H.: Harvey Lect. **32**: 194. 1937.
4. Houssay, B. A. and A. Biasotti: *Rev. Soc. argent. Biol.* **12**: 104. 1936; *C. R. Soc. Biol.*, Paris. **123**: 497. 1936.
5. Houssay, B. A. and A. Biasotti: *Rev. Soc. argent. Biol.* **9**: 29. 1933; *C. R. Soc. Biol.*, Paris, **113**: 469. 1933.
6. Houssay, B. A. and L. F. Leloir: *Rev. Soc. argent Biol.* **11**: 464. 1935; *C. R. Soc. Biol.*, Paris, **120**: 670. 1935.
7. Houssay, B. A. and A. Biasotti: *Rev. Soc. argent. Biol.* **14**: 308, 1938; *C. R. Soc. Biol.*, Paris, **129**: 1261, 1938.
8. Houssay, B. A., V. G. Foglia, and Dosne de Pasqualini Chr.: *Rev. Soc. argent. Biol.* **22**: 147. 1946.
9. De Bodo, R. C. and M. W. Sinkoff: *Ann. N.Y. Acad. Sci.* **57**: 23. 1953.
10. De Bodo, R. C. and M. W. Sinkoff: *Diabetes* **3**: 87. 1954.
11. Lazarus, S. S. and B. W. Volk: *Metabol. clin. Exper.* **1**: 355. 1952.
12. Houssay, A. B.: La acción diabetógena de la hipófisis, Thesis Fac. Med. Buenos Aires, 1945.
13. Houssay, B. A. and E. Anderson: *Rev. Soc. argent. Biol.* **25**: 91. 1949; *Endocrinology* **45**: 627. 1949; *C. R. Soc. Biol.*, París, **143**: 1269. 1949.
14. Houssay, B. A., R. R. Rodríguez and A. F. Cardeza: *Rev. Soc. argent. Biol.* **29**, 33, 1953; *C. R. Soc. Biol.*, París, **148**: 190. 1954.
15. Houssay, B. A., E. Anderson, R. W. Bates and Ch.H. Li: *Endocrinology* **57**: 55. 1955.
16. Sinkoff, M. W. and R. D. De Bodo: *Arch. exp. Path. Pharmak.* **219**: 100. 1953.
17. Hartmann, L. F.: *Rev. Soc. argent. Biol.* **30**: 7. 1954; *C. R. Soc. Biol.*, París, **148**: 1637. 1954.

EFFECT OF TESTOSTERONE ON CIRCULATING EOSINOPHILS IN THE GUINEA PIG

MALVINA SCHWEIZER

Department of Biology, Washington Square College of Arts and Science, New York University, New York, N.Y.

McDERMOTT, Fry, Brobeck and Long (1) interpreted the absence in the adrenalectomized castrate male rat of the eosinopenia following epinephrine which occurred in the adrenalectomized male to indicate "a slight overlap in the effect of adrenal and gonadal steroids." An eosinopenic effect of male sex hormone is also suggested by 1) the lower level of circulating eosinophils in the normal male than female guinea pig (2, 3, 4), and 2) the greater increase in circulating eosinophils after hypophysectomy in the male guinea pig than in the female (4). Calculation of the number of eosinophils per mm.^{3} from Crafts' data (5, 6) on the percentage eosinophils in the rat shows that in this species there is also a greater increase in the hypophysectomized male than female.

Direct evidence for an eosinopenic effect of testosterone in the guinea pig is given in the following report.

EXPERIMENTAL PROCEDURE

Adult male guinea pigs from our own colony were used in these experiments. Details of the care of these animals, necessary precautions in making the white blood cell counts and the method used for determining both the number of polymorphonuclear leucocytes and eosinophils by direct chamber counts are given elsewhere (7). The only modification in procedure was the use of three counting chambers (one Neubauer and two Levy, depth .2 mm.) for estimating the number of eosinophils.

Castration was performed on adult males (90–280 days old) using Nembutal anaesthesia. White blood cell counts were made just prior to and at monthly intervals after castration.

Two months after castration a group of animals was given daily subcutaneous injections of testosterone (Oreton-F)[1] in aqueous suspension (500 μg./100 gm. body weight) for one week. White blood cell counts were made before starting the injections, the day after the last injection, and at weekly intervals thereafter.

OBSERVATIONS

Data are summarized in Table 1.

Castration. One month after castration circulating eosinophils were 85% above the pre-castration level. Although the mean level of these cells was even higher 2 and 3 months after castration (+132% and +140%, re-

Received June 21, 1956.

[1] Kindly supplied by Dr. Milton Eisler, Schering Corporation, Bloomfield, N.J.

spectively) this additional rise is not statistically significant. The number of eosinophils in the castrate male is similar to that in the normal female (191 ± 34.7, Schweizer, 1955). The level of circulating polymorphonuclear leucocytes was not affected by castration.

Testosterone. The administration of testosterone daily for one week re-

TABLE 1

Experimental procedure	No. of animals	Polymorphonuclear leucocytes cells/mm.^{3} ±S.E.	Eosinophils cells/mm.^{3} ±S.E. (% change)	Eosinophils/1000 polymorphonuclear leucocytes (% change)
		Effect of Castration		
Controls				
Normal males	16	4395 ± 504	78 ± 9.8	17.7
Castrate:				
1 month	16	3254 ± 329	143 ± 26.8[2] (+ 84.6%)	43.9 (+148.0%)
2 months	12	3413 ± 391	175 ± 34.5[2] (+132.0%)	51.2 (+189.3%)
3 months	12	3533 ± 374	187 ± 26.8[2] (+139.7%)	52.9 (+198.8%)
		Effect of Testosterone[1]		
Controls:				
Castrate 2 months	8	3423 ± 304	180 ± 32.1	52.6
Time after last Injection:				
1 day	4	4000	58 (− 67.7%)	14.5 (− 72.4%)
1 week	8	4610 ± 652	62 ± 6.4[3] (− 65.5%)	13.4 (− 74.5%)
2 weeks	8	2894 ± 504	74 ± 17.9[3] (− 58.8%)	25.5 (− 51.5%)
3 weeks	8	3144 ± 514	131 ± 17.1 (− 27.2%)	41.6 (− 20.9%)
4 weeks	4	3560	142 (− 21.1%)	39.9 (− 24.1%)
6 weeks	4	3362	164 (− 8.8%)	50.2 (− 4.5%)
		Change with Age		
Age:				
17–26 days	11	1395 ± 170	84 ± 12.7	60.2
35–90 days	14	4859 ± 544[4]	68 ± 10.0 (− 19.0%)	14.0 (− 76.7%)
95–300 days	21	4300 ± 422[4]	81 ± 8.5 (− 3.3%)	18.9 (− 68.6%)

[1] Daily injections for one week.
[2] Highly significant difference from normal males, $p < .01$.
[3] Highly significant difference from castrate controls, $p < .01$.
[4] Highly significant difference from males 17–26 days old, $p < .01$.

duced the circulating eosinophils in the two-month castrate to levels characteristic of the normal male guinea pig. This effect persisted for two weeks after the termination of injections, but by the third week eosinophils were approaching the castration level. There was no significant alteration in the number of polymorphonuclear leucocytes as a result of the administration of testosterone to the castrate.

Age. The white blood cell counts of a group of normal males ranging in age from 17 to 300 days were analyzed to see whether the onset of sexual maturity was associated with a decrease in eosinophils. The data show no significant difference in the number of circulating eosinophils per mm.^{3} of blood in male guinea pigs 17–26 days old from those in the other age groups (35–90, and 95–300 days). This unexpected finding is clarified, however, when it is noted that polymorphonuclear leucocytes are more than

200% higher in the other age groups than in guinea pigs 17–26 days old. When the number of eosinophils is computed per thousand polymorphonuclear leucocytes (instead of per mm.^{3} of whole blood) the *proportion* of eosinophils in the sexually immature animals is similar to that found in the castrate and is more than 200% above that in the mature guinea pig. The validity of this method of reporting eosinophil levels when the total number of polymorphonuclear leucocytes increases is discussed below.

DISCUSSION

It was suggested elsewhere (8) that when there is a significant release of polymorphonuclear leucocytes from the bone marrow a peripheral eosinopenia may be masked by the concomitant addition of eosinophils to the the circulating blood. Under these conditions, the number of eosinophils per thousand polymorphonuclear leucocytes gives a more reliable picture of alterations in the level of eosinophils than does the number of these cells per mm.^{3} of whole blood. In the above experiments the per cent change in eosinophils is essentially the same whichever method of reporting is used so long as the polymorphonuclear count is constant (castration, and testosterone administration to the castrate) but the marked increase in polymorphonuclear leucocytes in the guinea pig after 30 days of age tends to mask the change in the level of circulating eosinophils with sexual maturity unless these cells are computed per thousand polymorphonuclear leucocytes.

The sex difference in the number of circulating eosinophils reported by various workers might be due to an eosinopenic effect of androgens, an eosinophilic effect of estrogens, or both. The above report demonstrates an eosinopenic effect of male sex hormone. The level of circulating eosinophils in the castrate male is comparable with that in the normal female, chronic administration of testosterone reduces this level in the castrate to that of the normal male, and in the sexually immature male the level is similar to that in the castrate. (Parallel studies on the female guinea pig are in progress.) In acute experiments on the adrenalectomized mouse Spiers and Meyer (9) found an increase in eosinophils 4 and 8 hours after injection of 750 μg. of testosterone propionate, and Halberg (10) found no eosinopenia 10 and 24 hours after implantation of 2.8 mg. pellets of testosterone propionate and methyl testosterone although adrenal steroids caused a maximal eosinopenia under comparable conditions. These reports suggest that testosterone may be producing its effect via the adrenal cortex.

In the above report it was also shown that some maturational factor necessary for the production or release of polymorphonuclear leucocytes is lacking in the very young (less than 30 days) guinea pig. This is, presumably, not a gonadal factor since neither castration nor administration of testosterone to the castrate altered the level of these cells. Fand (11) found

a lower total white blood cell count in the very young guinea pig but unpublished data from this laboratory show that the number of mononuclear cells is only slightly lower in the 17 to 26-day old guinea pig than in the older animal; that the lower white blood cell count of the immature animal is in large measure due to the low level of polymorphonuclear leucocytes.

SUMMARY

Chronic experiments on the male guinea pig demonstrate an eosinopenic effect of androgenic hormone. The level of circulating eosinophils is 1) increased in the male castrate to that characteristic of the normal female, 2) decreased in the male castrate following chronic administration of testosterone to that found in the normal male, and 3) as high in the sexually immature male (17–26 days old) as in the male castrate.

There is a marked (more than 200%) increase in the number of polymorphonuclear leucocytes in the guinea pig after 30 days of age which is evidently not due to gonadal maturation since the number of these cells is not affected either by castration or administration of testosterone to the castrate.

REFERENCES

1. McDermott, W. V., E. G. Fry, J. R. Brobeck and C. N. H. Long: *Yale J. Biol. and Med.* **23**: 52. 1950.
2. Dworetsky, M., C. F. Code and G. M. Higgins: *Proc. Soc. Exp. Biol. and Med.* **75**: 201. 1950.
3. Dews, P. B. and C. F. Code: *Proc. Soc. Exp. Biol. and Med.* **77**: 141. 1951.
4. Schweizer, M.: *Endocrinology* **56**: 693. 1955.
5. Crafts, R.: *Endocrinology* **29**: 596. 1941.
6. Crafts, R.: *Endocrinology* **39**: 401. 1946.
7. Schweizer, M.: *Endocrinology* **53**: 293. 1953.
8. Schweizer, M. Submitted for publication.
9. Speirs, R. S. and R. K. Meyer: *Endocrinology* **45**: 403. 1949.
10. Halberg, F.: *J. Pharm. and Exp. Therap.* **106**: 135. 1952.
11. Fand, I.: Ph.D. Dissertation, New York University, 1956.

ON THE NEPHROSCLEROTIC ACTION OF 2-METHYL-9-FLUOROCORTISOL, A HIGHLY ACTIVE ARTIFICIAL MINERALOCORTICOID[1]

HANS SELYE AND PIERRE BOIS

Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada

2-Methyl-9-fluorocortisol (2Me-9F-COL) was shown to possess extraordinarily intense mineralocorticoid but comparatively slight glucocorticoid activity, as judged by various tests (1). This compound, like other active mineralocorticoids (e.g., desoxycorticosterone and aldosterone), also exhibits a mutual antagonism with glucocorticoids (e.g., cortisone, cortisol) in the regulation of inflammation, anabolism and lymphatic-tissue development (2). On the other hand, the production by typical mineralocorticoids of renal damage and hypertension is not counteracted but rather enhanced by glucocorticoids (3, 4). It was to be expected, therefore, that a compound such as 2Me-9F-COL would produce marked renal lesions and hypertension by virtue of its strong mineralocorticoid potency, despite the fact that it also possesses some glucocorticoid activity. The experiments to be described here demonstrate that this is actually the case; indeed, 2Me-9F-COL proved to be the most potent nephrotoxic steroid among all those hitherto tested.

MATERIALS AND METHODS

In 40 female Sprague-Dawley rats, having an average initial body-weight of 50 gm. (range: 47–57 gm.), the right kidney was removed, after which they were immediately placed on 1% NaCl as a drinking fluid. This was done because earlier experiments had shown that unilateral nephrectomy and a high sodium intake greatly augment the nephrotoxic activity of mineralocorticoids.

The animals were then subdivided into four equal groups of ten rats each and treated with various corticoids, as indicated in Table 1. All steroids were administered in the form of microcrystal suspensions, subcutaneously, once daily in 0.2 ml. of water. Since the handling of the animals and the daily injections of steroids represented a certain amount of stress, we treated one group of controls (Group I) with 4-chlorocortisone acetate (4Cl-CONA), a close derivative of the corticoids which, at least in doses up to 2 mg./day, proved to be virtually devoid of any detectable biologic activity, as judged by our preliminary experiments. A second group of controls (Group II) received DCA, a known active mineralocorticoid, which was administered at the daily dose of 2 mg./day. We knew from previous experience that this amount is just sufficient to produce marked renal and cardiovascular damage within 20 days. In Group III we

Received June 21, 1956.

[1] This work was subsidized by the Medical Research Board, Office of the Surgeon General, Department of the Army, contract DA-49-007-MD-186.

TABLE 1. SUMMARY OF PRINCIPAL FINDINGS CONCERNING THE EFFECTS OF OVERDOSAGE WITH 2ME-9F-COL

Group	Steroid	Final body-weight (gm.)	Kidney		Heart		Periarteritis in pancreas	Pleural fluid & ascites	Fluid-Intake†	Adrenals (mg.)	Thymus (mg.)	Spleen (mg.)
			(mg.)	Lesions	(mg.)	Lesions						
I	4Cl-CONA 2 mg./day	116±3.9	1059±45.0	0	503±23.5	0	0	0	63±3.1	27.7±1.5	258±22.1	424±24.8
II	DOCA 2 mg./day	102±5.7	1454±84.5	1.2	553±14.9	1.4	0	0	121±3.7	28.4±0.9	210±28.6	529±42.1
III*	2Me-9F-COL First 100 μg., then 50 μg./day	58±3.5	848±27.3	3.0	260±10.3	1.0	0.3	2.2 Much edema	106±6.2	16.9±0.2	Not detectable	82±11.7
IV	2Me-9F-COL 20 μg./day	90±3.7	1390±90.1	3.0	502±29.1	2.0	3.0	0.9 Slight edema	113±4.6	30.6±1.4	97±27.1	345±50.1

* Animals in this group so severely damaged that they had to be killed after 15 days of treatment (5 days earlier than those of all other groups).
† Mean of last ten days.

started treatment with 100 μg./day of 2Me-9F-COL, assuming, on the basis of its high mineralocorticoid activity, that it would probably be much more nephrotoxic than DCA. However, it soon became evident that even this dose was much too high, as the animals became intensely edematous by the tenth day of treatment; hence, at this time, we diminished the dose to 50 μg./day. During the next three days three animals of this Group died, with extraordinarily marked subcutaneous edema and fluid accumulations in the pleura and the peritoneal cavity. Therefore this Group of rats had to be killed on the 16th day (after 15 daily injections), by which time virtually all animals were moribund. The animals of Group IV received 20 μg. of 2Me-9F-COL. Although these rats gradually also developed some subcutaneous edema and marked fluid accumulations in the large body cavities, all but one survived until the scheduled termination date of the experiment, on the 21st day after the first injection.

Throughout the experiment all animals were fed exclusively on "Purina Fox Chow." Their fluid-intake was measured daily, as an indicator of the polyuria characteristic of mineralocorticoid overdosage. Blood-pressure measurements were made on the tenth and the 20th day with the tail-cuff method.

In Table 1 the mean final body-weights and organ-weights are listed with their standard errors, together with the mean intensity of histologically demonstrable morbid changes in the kidneys, hearts and pancreatic arteries. These lesions, just as the fluid accumulations in the pleura and peritoneum, could not be measured quantitatively with any degree of accuracy and were hence expressed in an arbitrary scale of 0 to 3+. The figures given in the corresponding columns of the table were arrived at by adding up the total number of plusses and dividing this sum by the number of animals in each group.

The organs were dissected and fixed in Susa solution, immediately after killing the rats with chloroform, and weighed in the fixed state. The tissues were then embedded in paraffin, sectioned at 6 μ., and stained with the Hotchkiss-McManus PAS-technique (5).

RESULTS

Our principal findings are summarized in Table 1.

In agreement with expectations, the controls (Group I), which were treated with the virtually inert 4Cl-CONA, were essentially normal in every respect, when compared with untreated animals of our colony. On the other hand, the same dose of DCA and the much smaller doses of 2Me-9F-COL produced very pronounced morbid changes.

The *body-weight* gain was only insignificantly inhibited by DCA, but growth was almost completely suppressed by the higher dose of 2Me-9F-COL (Group III), and considerably diminished even by 20 μg./day of this compound (Group IV). This diminution of growth is all the more remarkable because the rats weighed only 50 gm. at the onset of the experiment and it is very difficult to suppress the growth of such small rats, even with the most potent stressor agents. Furthermore, as we have said, both the 2Me-9F-COL-treated groups exhibited marked accumulations of pleural fluid, ascites and more or less pronounced subcutaneous edema, which tended to mask the retardation of growth, as assessed by the final body-weight.

An evaluation of the organ-weights must take into account the great

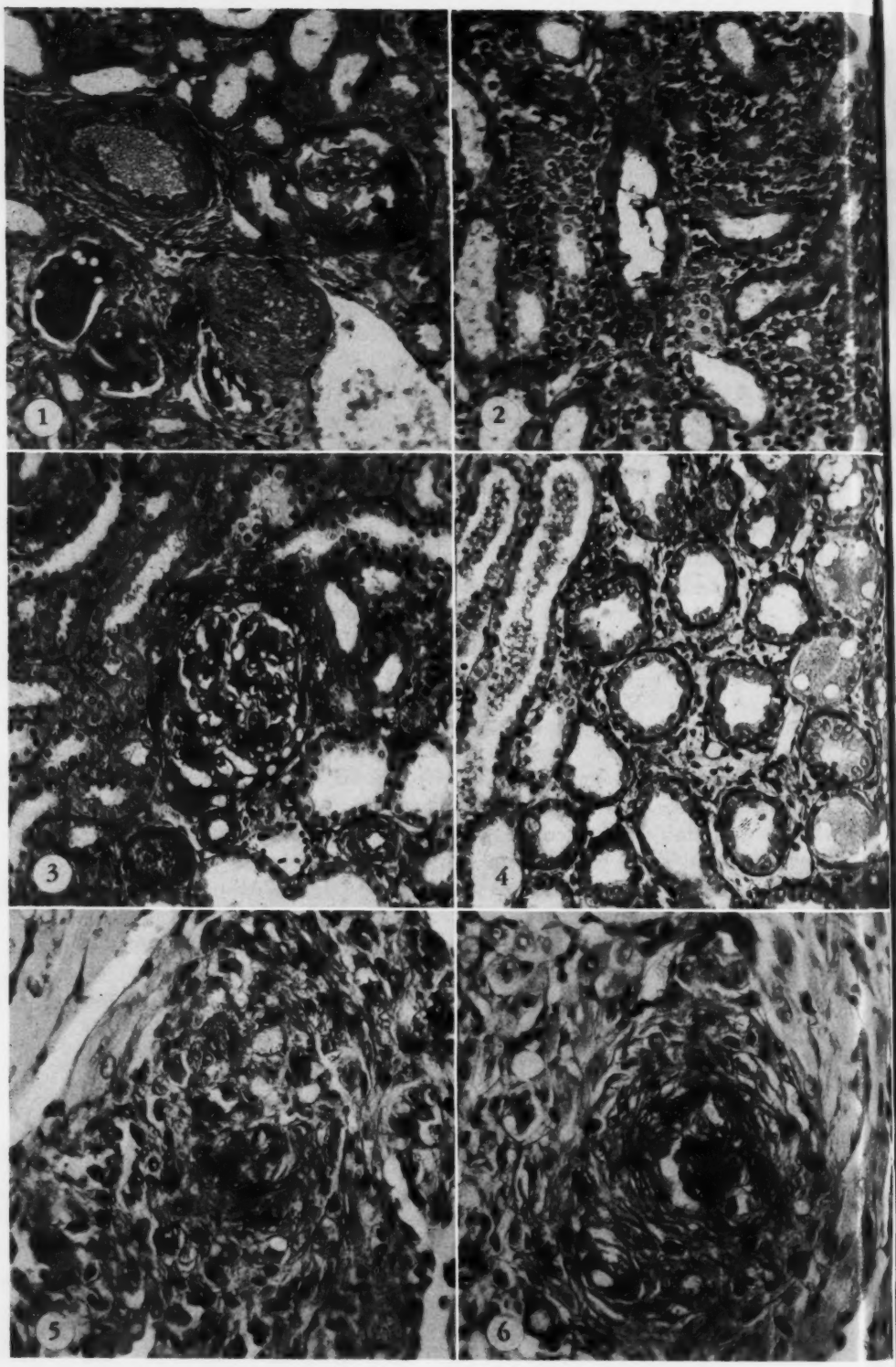
difference in the final body-weight of the various groups. It is not possible to do this satisfactorily by merely expressing organ-weights as a percentage of the total final body-weight, because growth retardation is not necessarily proportionate in all organs. Therefore the table lists the absolute organ-weights, which are instructive in themselves and may, in any event, be compared with the final body-weight. We attach much more importance, however, to the histologically demonstrable morbid lesions indicative of cardiovascular damage than to the weights of the organs.

The *kidney* underwent considerable hypertrophy (as compared to the 4Cl-CONA-treated controls) only in the rats receiving DCA or the small dose of 2Me-9F-COL. However, the relative weight of the kidney, expressed as a percentage of the body-weight, was also greatly augmented in the rats injected with the larger dose of 2Me-9F-COL, in which the final body-weight was only half that of the controls.

Histologically, the renal lesions were only of moderate intensity in the DCA-treated rats. There was some tubular dilatation and hyalinization of glomeruli, with the formation of more or less numerous hyalin casts. On the other hand, in both groups receiving 2Me-9F-COL, the renal changes were much more severe and have probably reached the maximum degree of intensity compatible with life. It will be recalled that the rats of Group III had to be sacrificed five days before all others, because of high mortality and the moribund condition of the survivors. It may be added that those of Group IV were also in very bad condition by the time of autopsy. This is perhaps why we were not able to distinguish any great difference in the intensity of the renal lesions in Groups III and IV.

The most characteristic histologic changes in the 2Me-9F-COL-treated rats are illustrated by microphotographs. The glomeruli underwent hyalinization and there were obvious signs of protein-leakage through the glomerular tufts, with proteinaceous, often strongly PAS-positive, casts in the tubules. Frequently, the glomeruli became very cellular and evidenced the morphologic characteristics of glomerular nephritis, sometimes with hemorrhages into Bowman's capsule (Figs. 1, 3, 4). In other animals —and sometimes even in other parts of the same kidney—the picture was more that of an interstitial nephritis, with round-cell infiltration of the stroma between the tubules and glomeruli (Fig. 2). Some of these round cells were very large and resembled immature blood-forming elements, but most of them were small lymphoid or plasma-cells; occasionally there were also polymorphonuclear leukocytes. In many places the basement membranes of the tubules were greatly thickened, strongly PAS-positive, and sometimes split up into lamellae (Fig. 4).

Perhaps the most striking change, however, was the extraordinarily widespread development of the "venous cushions," which we had repeatedly described as characteristic of rats developing particularly acute types


1
2
3
4
5
6

of nephritis or malignant nephrosclerosis. These cushions consist of a slightly PAS-positive interstitial substance and fibers with star- or spindle-shaped cells of the fibroblast type. They protrude into the lumina of larger veins and are rarely, if ever, observed in venules not accompanied by arteries (Fig. 1). Occasionally, there develop deeply PAS-positive coarse fibers or rods in the immediate vicinity of these cushions and sometimes their central parts undergo fibrinoid degeneration with the formation of deeply PAS-positive homogeneous material. Most of these venous cushions are lined by a regular endothelium, but occasionally their surface exulcerates and becomes the site of thrombus-formation. Although sometimes venous cushions of this kind are observed in rats heavily overdosed with DCA alone, they are most common when the syndrome of mineralocorticoid intoxication is particularly acute, due to simultaneous treatment with thyroxine (6) or calciferol (7). The development of these venous cushions is usually associated—as it was in the present experiment—with a great tendency to water-retention in the large serous cavities and the subcutaneous connective tissue.

The weight of the *heart* was much less characteristically affected by the steroids than that of the kidney; presumably because the experiment was not of sufficiently long duration to permit a marked secondary hypertrophy of the myocardium in response to the hypertensive disease. On the other hand, histologic lesions in the myocardium were well developed, both in the DCA and in the two 2Me-9F-COL groups. They consisted of periarteritis nodosa in the cardiac arteries and the formation of "Aschoff-body-like" circumscribed nodules, with the deposition of PAS-positive

---

Typical renal and cardiac changes produced by overdosage with 2Me-9F-COL. (All photographs illustrate organ changes produced by 20 μg. of the compound, as given in Group IV.)

FIG. 1. Characteristic "venous cushion," which protrudes into the cavity of a large vein near the lower right corner of the field. Note coarse intensely PAS-positive (here black) rods and fibers, just underneath the cushion, beginning hyalinization of a glomerulus, several deeply PAS-positive casts and a medium-size artery in the vicinity of the cushion-bearing vein (×190).

FIG. 2. Infiltration with various types of white blood-cells in the stroma of the kidney between tubules. Here the picture resembles an interstitial nephritis (×190).

FIG. 3. Leakage of PAS-positive plasma and fibrinoid material through the glomerular capillaries of a rather cellular glomerulus. The picture is reminiscent of glomerulonephritis (×190).

FIG. 4. Intense thickening of the strongly PAS-positive basement membranes around some tubules near the center of the field. The stroma is somewhat edematous but only slightly infiltrated with round cells (×400).

FIGS. 5 and 6. Two regions of the myocardium illustrating the development of the "Aschoff-body-like" granulomas. In Fig. 5 the center of the nodule is diffusely hyalinized and slightly PAS-positive. In Fig. 6 the center of the nodule is intensely PAS-positive and became completely homogeneous. It is surrounded by several nuclei and some cytoplasm but distinct cell-borders are not discernible, so that a hyalinized polynuclear giant-cell-like structure results (×400).

fibrinoid material surrounded by a granuloma which contained some polynuclear giant cells (Figs. 5 and 6).

Macroscopically detectable *periarteritis nodosa* in the mesenteric vessels was not seen in any group, although usually there is a great deal of it following more chronic treatment with DCA. Microscopically however—in addition to the periarteritis of the cardiac vessels which we have already mentioned—there was marked periarteritis nodosa in the pancreatic vessels of the animals receiving the smaller dose of 2Me-9F-COL, but only traces of arterial hyalinization were detectable in a few animals of the group receiving more heavy overdosage with this steroid. We shall come back to this apparently paradoxical finding in the discussion.

Fluid accumulations in pleura and peritoneum were extremely obvious in all the rats given the higher dose of 2Me-9F-COL; they were usually accompanied by a great deal of pitting subcutaneous edema. Indeed, some of the rats of this group exhibited a most singular "balloon-like" aspect, because of the extreme distention of their body cavities and subcutaneous tissue spaces with accumulated water. Neither the control rats nor those which developed the more chronic nephrosclerotic type of renal lesion under the influence of DCA showed any macroscopically detectable fluid-retention.

The *fluid-intake*, which served as a measure of polyuria in these experiments, was greatly augmented by DCA and by both dose-levels of 2Me-9F-COL. This fact becomes particularly evident if the mean daily volume of ingested fluid is considered as a percentage of the final body-weight. Interestingly, just when massive water-retention became clinically obvious in the 2Me-9F-COL groups, the water-intake tended to fall. This may have been due to a terminal drop in blood-pressure which, by diminishing filtration pressure, could decrease the fluid turnover.

It should be mentioned, in this connection, that *blood-pressure* measurements on the tenth and the 20th day of the experiment showed marked hypertension (up to 220 mm. Hg) in several animals of Groups II, III and IV, but since severe systemic damage by the 2Me-9F-COL resulted in the eventual collapse of the blood-pressure in many animals, these determinations were too variable to be represented in the table by a mean figure with a standard error.

The *adrenals* did not undergo marked compensatory atrophy in the rats treated with DCA or the small dose of 2Me-9F-COL, presumably because the specific ACTH-secretion-inhibiting effect of these corticoids was overcompensated by the stimulation of ACTH-secretion due to the stressor action of the cardiovascular and renal damage. However, in Group III, the adrenal atrophy was highly significant, probably because, at this dose-level, the inhibition of ACTH-secretion due to the specific actions of the corticoids overcompensated for the converse, nonspecific, stimulating effect of the resulting systemic stress.

The *thymus* and *spleen* underwent particularly pronounced involution as a result of 2Me-9F-COL treatment. This was to be expected, in view of the glucocorticoid potency of this steroid. DCA, on the other hand, produced no splenic and little thymic atrophy, since it is devoid of glucocorticoid potency. Even the slight thymic involution observed in the DCA-treated rats under these conditions was presumably due to endogenous glucocorticoids secreted under the influence of stress since, in adrenalectomized rats, DCA has no such effect.

DISCUSSION

The principal result of these observations is that, in rats suitably sensitized to the nephrotoxic actions of mineralocorticoids, 2Me-9F-COL proved itself more than 100 times as active as DCA in producing cardiovascular damage, fluid-retention and hypertension. As far as can be gathered from our personal observations and from the literature which deals with this topic, 2Me-9F-COL is therefore the most potent steroid in this respect.

The apparently paradoxical fact that 2Me-9F-COL produces marked periarteritis nodosa in the pancreatic vessels at low but not at high dose-levels deserves further comment. This finding is reminiscent of our observations concerning the anticortisol effect of 2Me-9F-COL (2). Those experiments had shown that small doses of this artificial corticoid antagonize the antiphlogistic effect of cortisol in the "granuloma-pouch" test more effectively than large doses. Such findings are quite in agreement with what has been termed the "law of intersecting dose-effect curves." It had been found that, when a solution containing fixed proportions of cortisol acetate (COLA) and DCA is administered to adrenalectomized rats, the cortisol actions (catabolism, inhibition of exudation, thymolysis, splenic atrophy) predominate at high, and the opposite, desoxycorticosterone-type of activity predominates at low dose-levels. This was ascribed to the fact that the DCA-activity rises rapidly to its optimum level, but then a "ceiling" is reached and raising the dose further fails to increase the effect. The cortisol-type of activity, on the other hand, rises more slowly but does not flatten out until it far exceeds the ceiling of its antagonist (4, 8).

Since 2Me-9F-COL is known (1) to possess considerable glucocorticoid activity (about 39 times that of cortisol), in addition to its much more evident mineralocorticoid action (about 90 times that of DCA), it was to be expected that small doses of the compound would be prophlogistic, anticatabolic and stimulating to thymic and splenic development, while large doses would act inversely. This may well explain the virtual absence of periarteritic changes in the pancreas of the rats receiving the higher dose of 2Me-9F-COL, because pancreatic periarteritis nodosa is known to be subject to the "law of interescting dose-effect curves." On the other hand, the nephrotoxic effect of mineralocorticoids is further aggravated by

glucocorticoids at all dose-levels at which the interaction between these hormones has been hitherto explored (2, 4). Cardiac periarteritis nodosa assumes an intermediate position in this respect, perhaps because it depends not only upon the balance between prophlogistic and antiphlogistic corticoids (that is, mineralo- and glucocorticoids), but also upon the intensity of renal damage. The latter acts rather selectively upon the cardiac vessels, perhaps through the intermediary of the renal-pressor mechanism.

Despite its strong mineralocorticoid and comparatively weak glucocorticoid effect, 2Me-9F-COL caused pronounced inhibition of body-growth, thymus-atrophy and splenic involution. This is likewise in accord with the "law of interescting dose-effect curves" because, as we saw, in all thesere spects, in mixtures of gluco- and mineralocorticoids, the former predominate over the latter at high dose-levels (4). Of course, the same is true when both types of corticoid activities are exhibited by the same molecule, as is the case with 2Me-9F-COL.

SUMMARY

Experiments on rats—sensitized to the nephrotoxic actions of mineralocorticoids by unilateral nephrectomy and a high NaCl intake—indicate that 2-methyl-9-fluorocortisol (2Me-9F-COL) is more than 100 times as active as desoxycorticosterone acetate (DCA) in producing cardiovascular damage, fluid-retention and hypertension.

Among the changes produced by acute heavy overdosage with 2Me-9F-COL, the rapid development of fluid accumulations in the large serous cavities and the subcutaneous tissue are especially prominent. At the same time, there are renal lesions, characteristic of glomerular and interstitial nephritis, accompanied by the particularly widespread development of "venous cushions" in the renal veins. These changes differ from the malignant nephrosclerosis elicited by more chronic overdosage with DCA.

Acknowledgment

The authors gratefully acknowledge generous supplies of 4-chlorocortisone acetate from British Drug Houses Limited, desoxycorticosterone acetate ("Cortate") from Schering Corporation, and 2-methyl-9-fluorocortisol from The Upjohn Company.

Addendum

Since this manuscript went to press we were able to show that the chloro analogue of 2Me-9F-COL, administered in the form of its acetate [2-methyl-9(α)-chlorocortisol acetate], possesses an even greater mineralocorticoid activity than the fluoro compound. As judged by its anticortisol effect, tested with the granuloma-pouch technique, it is approximately 200 times as active as DCA (Selye, H. and P. Bois, J. Clin. Endocrinol., in press), while its nephrotoxic action, in unilaterally nephrectomized rats given 1% NaCl as a drinking fluid, is approximately 400 times that of DCA (Selye, H. and P. Bois, Canad. M.A.J., Nov., 1956).

REFERENCES

1. Byrnes, W. W., L. E. Barnes, B. J. Bowman, W. E. Dulin, E. H. Morley and R. O. Stafford: *Proc. Soc. Exper. Biol. and Med.* **91:** 67. 1956.
2. Selye, H. and P. Bois: *Proc. Soc. Exper. Biol. and Med.* **92:** 362. 1956.
3. Selye, H.: *Stress* Acta, Inc., Med. Publ., Montreal, 1950.
4. Selye, H.: *Science* **121:** 368. 1955.
5. McManus, J. F. A.: *Am. J. Path.* **24:** 643. 1948.
6. Selye, H. and P. Bois: *Proc. Soc. Exper. Biol. and Med.* **92:** 164. 1956.
7. Selye, H. and P. Bois: *A.M.A. Arch. Path.* Münch. Med. Wchnschr. **98:** 1015. 1956.
8. Selye, H. and P. Bois: In: *Fourth Annual Report on Stress* (H. Selye and G. Heuser), pp. 533–552. Acta, Inc., Med. Publ., Montreal, 1954.

THE RELATION OF THE ADRENAL TO PHOSPHORUS METABOLISM OF BONE MARROW[1,2]

IRWIN BERMAN,[3] ABRAHAM EDELMANN[4] AND ALBERT S. GORDON

Departments of Biology, Graduate School of Arts and Science, New York University, New York City, and Brookhaven National Laboratory, Upton, Long Island

IT HAS been demonstrated amply (1–5) that the endocrine secretions serve a regulative function in hematopoiesis. Those studies (1–3) relating to the adrenals have indicated significant alterations in the numbers and types of bone marrow elements as a consequence of adrenalectomy. Replacement treatment in the form of adrenal cortical extracts or the 11-oxygenated steroids prevents the altered marrow cytology found in the adrenal-insufficient rat (3, 6).

In an effort to determine the mechanisms that underlie these morphologic changes attention has turned to studies of the effects of adrenalectomy and of adrenal cortical factors on the chemical composition and metabolism of bone marrow. In this regard, significant increases in aerobic and anaerobic glycolysis have been noted in marrow obtained from rats at 5 days after adrenalectomy (7). It has been reported also (8) that the concentrations of ribosenucleic acid (RNA) and deoxyribosenucleic acid (DNA) within the marrow of the rat decrease slightly at 3 and 5 days after adrenal removal and that chronic administration of corticotrophin to intact animals will also depress the nucleic acid levels within this organ (9).

The present report is concerned with a study of the influence of adrenalectomy on several parameters of phosphorus metabolism in bone marrow. Both the concentration within and the incorporation of radioactive inorganic phosphorus (P^{32}) into the phospholipid (PL), DNA, and RNA phosphorus fractions of rat bone marrow were assessed at four time intervals after adrenal removal.

Received June 25, 1956.

[1] Taken from a thesis presented by Irwin Berman to the Faculty of the Graduate School of Arts and Science, New York University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology.

[2] Research carried out at Brookhaven National Laboratory, Upton, L. I. Supported by grants-in-aid from the National Science Foundation and from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

[3] Present address: Department of Radiology, Stanford University School of Medicine, San Francisco, California.

[4] Present address: Nuclear Science and Engineering Corporation, Pittsburgh, Pennsylvania.

The effects of Lipo-adrenal cortex extract (Upjohn and Co.) and two dosage levels of 17-hydroxycorticosterone (free alcohol) upon the various P fractions of marrow were also studied in the adrenalectomized rat.

METHODS

Adult male rats of the Carworth Farms strain, weighing 185–200 gm. were used in all the experiments. The animals were kept in a room maintained at a temperature of 23° ± 1° C and were allowed food (Purina Chow) and water *ad libitum*. Both the adrenalectomies and sham adrenalectomies were performed using the dorsal approach while the animals were under ether anesthesia. For the adrenalectomized animals 1% saline was substituted for drinking water. Adrenalectomized and appropriate control groups (sham adrenalectomized and unoperated animals) were sacrificed at 1, 5, 10 and 14 days after their respective operations. Approximately 18 hours prior to the killing of the animals, all solid food was removed from the cages.

Two hours before sacrifice, each animal received a standard 0.5 ml. intraperitoneal injection of 100 μc. (approximately 0.5 μc./gm. body weight) of radioactive phosphorus in the form of $Na_2HP^{32}O_4$. At the end of the experimental periods, the animals were anesthetized lightly with ether, partially exsanguinated by cardiac puncture, brought into a cold room (0° to 4° C) and decapitated to complete the exsanguination process. The methods for obtaining the bone marrow from the tibias and femurs have been published previously (10). The marrow was placed in tared centrifuge tubes and the wet weights determined. The samples were brought to a constant volume by the addition of physiological saline and a homogeneous suspension was made by gently drawing the marrow up and down in a drawn-out rubber-bulbed glass pipette, the ends of which were fire-polished to minimize cellular damage. Aliquots were then taken for the separation of the PL, RNA, and DNA phosphorus fractions of the marrow and for the determination of the total nucleated cell counts of the suspensions. The cell counts were made by the chamber method, using the improved Neubauer hemocytometer. The diluting fluid was 0.33% acetic acid and all counts were required to agree to within ±10%.

The combined methods of Schmidt and Thannhauser (11) and Schneider (12) were used to separate the bone marrow into its component phosphorus fractions. In certain tissues, the procedure indicated does not yield a RNA fraction with a high degree of radiochemical purity (13). However, recent analysis (14) of bone marrow by methods (13) which assure an uncontaminated RNA fraction yield activities that differ from those obtained in the present work by no more than 10%. The procedures, except for those involving the extraction of the phospholipids, were carried out in the cold room (2–4°). The final solutions of the several phosphorus fractions were transferred quantitatively to calibrated test tubes, evaporated to dryness, and digested with concentrated sulphuric acid. The digest was boiled with water to hydrolyze pyrophosphate. The tubes were then diluted to 10 ml. with additional water following which aliquots were taken for phosphorus analysis and for counting. In this fashion all of the phosphorus fractions were reduced to the same final form and the phosphorus content (15) and radioactivity of the same solution were determined.

Two 1-ml. aliquots were taken from each acid digest and prepared for radioactive analysis by pipetting them into stainless steel cups (1″ in diameter and 3/16″ high). The acid was neutralized by the dropwise addition of concentrated ammonium hydroxide and the material dried at 60° C. By this method a thin even precipitate was formed which weighed 8 mg./cm.^{2}. This value proved well below the level (20 mg./cm.^{2}) at which loss of counts occurs from self absorption.

Four standards of the amount of P^{32} injected into the animals were prepared by

diluting the actual amount of material injected to such a level as to insure a moderately high counting rate. These standards were prepared each time a group of animals was sacrificed, and were counted along with each fraction assayed for radioactivity. In this manner, corrections for the decay of phosphorus were avoided regardless of the time interval between the counting of the different fractions obtained from each animal. Each planchette was counted in triplicate and for such time as was required to reduce the statistical error of counting to less than $\pm 3\%$.

The radioactivity of each sample and standard was determined with a Geiger-Müller tube. The amount of radioactive phosphorus which was incorporated into each fraction was expressed as the specific activity of the fraction corrected for body weights and for the actual amount of P^{32} injected. This value was calculated by the following formula:

$$\text{S.A.} = \frac{P^{32} \text{ counts per min. (in the fraction)/mg. } P^{31} \text{ (in the fraction)} \times 100}{P^{32} \text{ counts per min. injected/grams of body wt.}}$$

The phosphorus content of the fractions is reported as mg. phosphorus per gm. of fresh tissue weight (wet weight) and $\mu g. \times 10^7$ per cell.

The specific activity of plasma inorganic phosphorus at 0.5, 2, 4, 8, 12 and 16 hours after injection of P^{32} into 5-day adrenalectomized and control animals was determined by the isobutyl alcohol benzene extraction method of Berenblum and Chain (16) as modified for radioactive analysis by Ernster, Zetterstrom and Lindberg (17).

All hormonal restoration studies were conducted on 5-day adrenalectomized rats. The animals were primed daily for 2 days prior to adrenalectomy with subcutaneous injections of their respective factors (vide infra). Adrenalectomies were performed on the 3rd day and treatment was continued on this day and daily for 4 subsequent days. One group of animals received 0.5 ml. injections of Lipo-adrenal cortex extract, the controls for which were injected with 0.5 ml. quantities of cottonseed oil, the vehicle for the extract. Those animals which were treated with 17-OH corticosterone received either 0.5 mg. or 4.0 mg. of this steroid suspended in 1 ml. physiological saline; controls for these groups received injections of the saline.

For all data the significance of differences between the mean values of experimental and control groups are based on probability values (P) calculated from the distribution of Fisher's "t" (18). Those P values greater than 0.05 are considered not significant. Values falling between 0.05 and 0.01 are accorded significance, whereas values below 0.01 are termed highly significant. Only those animals which on autopsy revealed completeness of adrenal removal have been included in the statistical analysis of the data.

RESULTS

The specific activities of the DNAP, RNAP and PLP fractions of bone marrow at the 4 time intervals after adrenal removal are presented in Figure 1. The control group (1A) to which these values are compared is a composite one, comprising animals sham adrenalectomized and sacrificed at 1, 5, 10 and 14 days after the operation. Since statistical analysis revealed that the specific activities of the three fractions in the four sham series did not differ significantly from each other or from a series of unoperated animals, the respective data were pooled and treated as a single control to which each of the adrenalectomized groups was compared. It is evident from the data that by 24 hours after adrenalectomy a marked increase in the incorporation of P^{32} occurred in the DNA ($P < 0.01$) and RNA ($p < 0.05$) fractions of the bone marrow. However, at 5 days after

the operation the specific activity of DNAP ($P < 0.01$), RNAP ($P < 0.05$) and PLP ($P < 0.05$) were below control levels. At 10 days after adrenalectomy the incorporation of the isotope into DNA was still below ($P < 0.01$) that of the control, whereas the specific activity of RNAP and PLP had increased somewhat, and were now neither significantly different from the previous group (5-day adrenalectomized) nor from the control

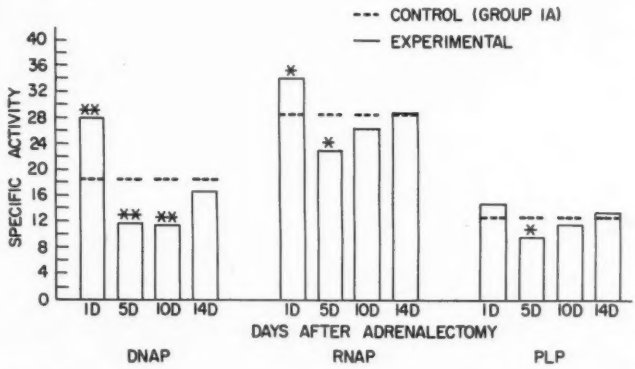


FIG. 1. Effects of adrenalectomy upon the specific activity of deoxyribosenucleic acid phosphorus (DNAP), ribosenucleic acid phosphorus (RNAP) and phospholipid phosphorus (PLP) in rat bone marrow. 1D, 5D, 10D and 14D refer to the results obtained at 1 day, 5 days, 10 days and 14 days after adrenalectomy. Asterisks over bars represent significant differences from control (Group 1A) values (dashed lines). One asterisk indicates $P < 0.05$. Two asterisks indicate $P < 0.01$.

animals. By the 14th day the incorporation of P^{32} into all fractions had been restored to control levels.

Figure 2 represents the results obtained in adrenalectomized rats with the two forms of hormonal treatment. Both Lipo-adrenal cortex extract and 17-OH corticosterone, in the two dosage levels employed, were effective in preventing the decrease in the incorporation of P^{32} which was observed in the DNA fraction at 5 days after adrenal removal. Of the two groups receiving 17-OH corticosterone those which received 0.5 mg. of the steroid displayed the more marked rise in DNAP specific activity. While 17-OH corticosterone in the lower dose increased the specific activity of the RNAP fraction ($P < 0.05$) of the marrow, the considerable rise (45%) in specific activity of the PLP fraction did not prove to be of significance (P between 0.10 and 0.05). Neither Lipo-adrenal cortex nor the higher dose of 17-OH corticosterone increased the specific activity of the PLP or RNAP fractions.

A summary of the data obtained for the phosphorus content of the three bone marrow fractions is given in Table 1. Of all the experimental groups studied those animals which received 4.0 mg. doses of 17-OH corticosterone

showed the greatest departure from the vehicle-injected controls. This dosage of hormone increased the DNAP content per cell, while it decreased the RNAP and PLP of the marrow on a weight basis. Neither Lipo-adrenal cortex nor 17-OH corticosterone in the lower dose had any action on the phosphorus content of the marrow. The only effect produced by adrenal-

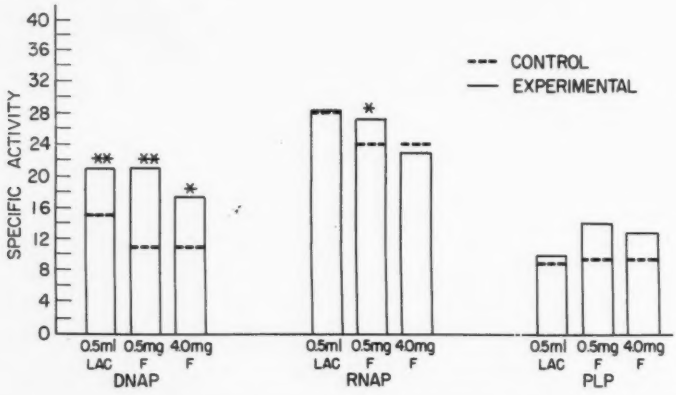


FIG. 2. Effects of replacement treatment upon the specific activity of deoxyribosenucleic acid phosphorus (DNAP), ribosenucleic acid phosphorus (RNAP) and phospholipid phosphorus (PLP) in the bone marrow of the 5 day adrenalectomized rat. LAC-Lipo-adrenal cortex extract; F-17-OH corticosterone. Asterisks over bars represent significant differences from adrenalectomized control values (dashed lines). One asterisk indicates $P < 0.05$. Two asterisks indicate $P < 0.01$.

ectomy was a decrease in RNAP (per cell basis) observed at 5 days and at 10 days (wet weight basis) after the operations.

The specific activity time curves for plasma inorganic P in the adrenalectomized and control animals are shown in Figure 3. The difference between the experimental and control values observed at 0.5 hours was significant. However, at 2 hours after the administration of P^{32} this change was no longer apparent and no further differences in the specific activities of plasma inorganic phosphate were noted.

DISCUSSION

The present studies make it clear that there is a time response relationship for the effects which adrenal steroid withdrawal induces in the phosphorus metabolism of bone marrow. At one day after adrenalectomy, significant increases were noted in the incorporation of P^{32} into the RNAP and DNAP fractions of the marrow. These results cannot be attributed to the effects arising from the operative procedures, as no difference was observed in the specific activity of these fractions in the marrow of one-day sham adrenalectomized and unoperated rats. In view of the prevailing

TABLE 1. CONCENTRATION AND CELLULAR CONTENT OF DNAP, RNAP AND PLP IN RAT BONE MARROW (MEANS ± S.E.)

Group	No. of animals	DNAP		RNAP		PLP	
		mg./gm. wet wt.	μg. $\times 10^7$/cell	mg./gm. wet wt.	μg. $\times 10^7$/cell	mg./gm. wet wt.	μg. $\times 10^7$/cell
Control	19	1.58 ± 0.04	8.10 ± 0.67	0.94 ± 0.03	4.63 ± 0.66	0.61 ± 0.02	2.97 ± 0.18
1D[1] ADX.	8	1.46 ± 0.07	7.41 ± 0.70	1.05 ± 0.10	5.27 ± 0.58	0.61 ± 0.03	3.19 ± 0.36
5D ADX.	12	1.56 ± 0.03	8.12 ± 0.68	0.90 ± 0.07	3.38 ± 0.46*	0.56 ± 0.03	2.62 ± 0.43
10D ADX.	8	1.60 ± 0.03	7.43 ± 0.45	0.77 ± 0.06*	4.01 ± 0.28	0.61 ± 0.05	2.70 ± 0.29
14D ADX.	8	1.62 ± 0.03	9.23 ± 0.09	0.95 ± 0.03	5.74 ± 0.30	0.61 ± 0.07	2.89 ± 0.16
5D ADX + 0.5 ml. Lipo-adrenal cortex	8	1.65 ± 0.05	8.01 ± 0.45	0.68 ± 0.05	5.09 ± 0.98	0.56 ± 0.02	2.81 ± 0.29
Control (5D ADX. + 0.5 ml. cottonseed oil)	9	1.51 ± 0.13	7.48 ± 0.70	0.80 ± 0.06	4.15 ± 0.37	0.50 ± 0.04	2.39 ± 0.13
5D ADX. + 0.5 mg. F.	8	1.28 ± 0.06	6.19 ± 0.27	0.88 ± 0.04	4.61 ± 0.23	0.45 ± 0.03	2.18 ± 0.17
5D ADX. + 4.0 mg. F.	8	1.22 ± 0.03	7.68 ± 0.68*	0.71 ± 0.05*	4.55 ± 0.62	0.31 ± 0.03*	2.05 ± 0.29
Control (5 Day ADX. + 1.0 ml. Saline)	7	1.34 ± 0.05	5.71 ± 0.23	0.93 ± 0.05	4.30 ± 0.16	0.53 ± 0.04	2.24 ± 0.16

[1] D refers to numbers of days after adrenalectomy (ADX.).
* Indicates probability values $P < 0.05$ for means compared to the controls.

concept that the incorporation of P^{32} into DNA reveals the extent of mitosis in a tissue (19, 20, 21), it is likely that these results reflect an increased mitotic activity of some cellular type(s) within the marrow. This possibility is strengthened by other work in which increased incorporation of P^{32} into RNA and DNA have been shown to accompany an increase in mitotic activity of tissues (19, 22, 23).

In contrast to the observations in the one-day adrenalectomized rat was the complete reversal in the pattern of P^{32} incorporation found at 5 days

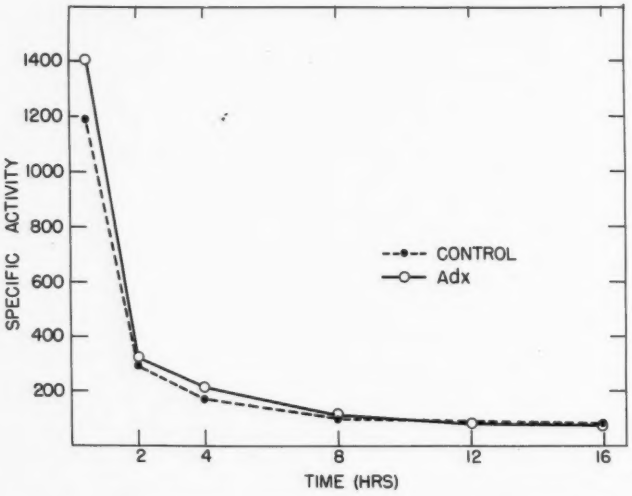


FIG. 3. The specific activity of plasma inorganic phosphate in 5 day adrenalectomized and control rats at different times after the administration of P^{32}.

after adrenal removal. At this time, significant decreases were noted in the specific activity of the PLP, DNAP and RNAP fractions of the marrow. It is conceivable that this decreased incorporation is related to the significantly decreased numbers and production of erythroid and possible neutrophilic elements noted in the marrow at 5 days after adrenalectomy (6). It is evident that the effects of adrenalectomy upon P^{32} incorporation are not the result of different plasma specific activities since the data indicate that at 2 hours after the administration of the isotope the specific activity of plasma inorganic phosphorus is identical in the adrenalectomized and control groups.

That additional mechanisms may operate is suggested in the results of the 10 and 14 day studies. At ten days after adrenalectomy the decreased incorporation of P^{32} into the DNA fraction was still evident, although its incorporation had increased in the PL and RNA fractions. By fourteen days after the operation the P^{32} uptake of all fractions had returned to

control levels. It is possible, therefore, that in all cells of the bone marrow the lack of adrenal hormones resulted in a temporary inhibition of RNA and PL synthesis or turnover with subsequent recovery, even though the decreased incorporation of P^{32} into DNA at ten days would still suggest a decreased mitotic activity in the bone marrow. The possibility that adaptive reactions are taking place at ten and fourteen days after adrenalectomy must also be considered. Autopsy of these animals proved the absence of adrenal tissue. It has been shown that the increased glycolysis in the marrow of the five-day adrenalectomized animal is restored almost to normal at fourteen days after the operation (7).

Although few alterations were noted in the concentration of phosphorus in the various fractions of the marrow, the significant decrease in the cellular content of RNAP observed at five days after adrenalectomy harmonizes with the results of Mandel et al. (8), although their finding that DNAP also decreases is not confirmed by the present work. It must be pointed out, however, that the lack of effect of adrenalectomy on the content of DNAP, both on a cellular and wet weight basis, which was observed in these experiments, is in accord with the report (6) that the total numbers of cells within the marrow remain unaltered after adrenalectomy and that the value for the DNAP content per cell obtained for the control animals (unoperated and sham adrenalectomized) is in good agreement with that obtained by Thompson *et al.* (24).

The experiments on hormonal-replacement treatment emphasize that the end result of hormonal administration is conditioned largely by the type and dosage of hormone given. The 0.5 mg. doses of 17-OH corticosterone were most effective in preventing the decreased P^{32} incorporation noted at five days after adrenalectomy.

This dose of hormone will restore the altered marrow picture in adrenalectomized rats to a state which most closely approximates that of the unoperated controls (6). One-half ml. doses of Lipo-adrenal cortex increased the P^{32} uptake of DNAP to normal levels but did not affect the P^{32} uptake of the PL fraction. Although Lipo-adrenal cortex increased the P^{32} uptake of the RNAP, this same effect was also noted in the RNAP fraction of the control animals which received cottonseed oil, the suspension medium for Lipo-adrenal cortex.

Of considerable interest were the effects which the 4 mg. doses of 17-OH corticosterone produced on the phosphorus metabolism of the bone marrow. Although this dose of steroid increased the incorporation of P^{32} into DNA, it was not as effective as the 0.5 mg. doses, and seemed to be without influence on the specific activity of RNAP and PLP. However, decreases in the phosphorus concentration were obtained in the latter fractions. It has been reported (6) that 4 mg. quantities of 17-OH corticosterone produce a marked erythropoiesis associated with a striking eosinopenia and lymphopenia within rat marrow. It would seem likely that the pattern of

phosphorus metabolism observed in the 4 mg. 17-OH corticosterone series is the resultant of the stimulatory and depressive effects which this large quantity of steroid exerts upon the cellular types of the marrow.

SUMMARY

An examination has been made of the effects of adrenalectomy on some aspects of bone marrow phosphorus metabolism in the rat. The incorporation of P^{32} into RNA and DNA was increased in the marrow of the one day adrenalectomized rat. Five days after adrenal removal the incorporation of P^{32} into the RNA, DNA and PL fractions was decreased. At 10 days the decreased specific activity of the DNAP was still evident although the specific activity of RNAP and PLP had increased to control levels. The incorporation of P^{32} into all fractions was restored to normal values by 14 days after adrenal removal.

One-half mg. doses of 17-OH corticosterone were most effective in preventing the changes noted in the 5-day adrenalectomized animal. Four mg. doses of this steroid increased the specific activity of DNAP but not RNAP or PLP. However, decreases in the concentration of phosphorus in the latter two fractions were observed. One half ml. injections of Lipoadrenal cortex extract also increased the incorporation of P^{32} into the DNA fraction of the marrow.

The specific activity time curves of plasma inorganic phosphorus were essentially the same in control and 5-day adrenalectomized rats.

It is suggested that these alterations in P^{32} incorporation are the result of changes in the morphology and mitotic activity of the bone marrow which are induced by adrenal removal and replacement treatment.

REFERENCES

1. Piliero, S. J., D. Landau and A. S. Gordon: *Science* **112**: 559. 1950.
2. Crafts, R. C.: *Endocrinology* **29**: 596. 1941.
3. Gordon, A. S., S. J. Piliero and D. Landau: *Endocrinology* **49**: 497. 1951.
4. Daughaday, W. H., R. H. Williams and G. A. Daland: *Blood* **3**: 1342. 1948.
5. Landau, D. and A. S. Gordon: *Endocrinology* **51**: 157. 1952.
6. Fruhman, G. J. and A. S. Gordon: *Endocrinology* **57**: 711. 1955.
7. Gordon, A. S.: *Recent Progress in Hormone Research* **10**: 339. 1954.
8. Mandel, P. M., P. Metais and R. Voegtlen: *Compt. Rend. Soc. Biol.* **147**: 1282. 1953.
9. Robbins, G. P., J. A. D. Cooper and H. I. Alt: *Endocrinology* **56**: 161. 1955.
10. Berman, I. and A. S. Gordon: *Am. J. Physiol.* **173**: 184. 1953.
11. Schmidt, G. and S. J. Thannhauser: *J. Biol. Chem.* **161**: 83. 1945.
12. Schneider, W. C.: *J. Biol. Chem.* **164**: 747. 1946.
13. Davidson, J. N. and R. M. S. Smellie: *Biochem. J.* **52**: 599. 1952.
14. Berman, I.: Unpublished Data.
15. Fiske, C. H. and Y. Subbarow: *J. Biol. Chem.* **66**: 375. 1925.
16. Berenblum, I. and E. Chain: *Biochem. J.* **32**: 295. 1938.
17. Ernster, L., R. L. Zetterstrom and O. Lindberg: *Acta. Chem. Scand.* **4**: 942. 1950.

18. FISHER, R. A.: Statistical Methods for Research Workers. Oliver and Boyd, Ltd., Edinburgh and London, 10th ed. 1948.
19. HULL, W. AND D. L. KIRK: *J. Gen. Physiol.* **33:** 335. 1950.
20. OSGOOD, E. E., J. G. LI, M. L. DUERST AND A. J. SEAMAN: *Science* **114:** 95. 1951.
21. LEBLOND, C. P., C. E. STEVENS AND R. BOGORCH: *Science* **108:** 531. 1948.
22. GERARDE, H. W., M. JONES AND T. WINNICK: *J. Biol. Chem.* **196:** 69. 1952.
23. JOHNSON, R. M. AND S. ALBERT: *Arch. Biochem. and Biophys.* **34–35:** 340. 1952.
24. THOMSON, R. Y., F. C. HEAGY, W. C. HUTCHINSON AND J. N. DAVIDSON: *Biochem. J.* **53:** 460. 1953.

CHANGES IN BLOOD ACTH UNDER VARIOUS EXPERIMENTAL CONDITIONS STUDIED BY MEANS OF A CROSS-CIRCULATION TECHNIQUE[1,2]

A. BRODISH[3] AND C. N. H. LONG

Department of Physiology, Yale University, New Haven, Connecticut

IN PREVIOUS papers (1, 2) a method has been described for the estimation of ACTH in blood by the establishment of a crossed circulation between an intact and hypophysectomized rat. By this means, the ACTH activity in 10 ml. of blood of less may be detected. Since this method does not involve any chemical manipulation of the blood prior to the assay of ACTH by the adrenal ascorbic acid depletion technique, it offers possibilities to determine the effect of various procedures on the ACTH secretion from the pituitary of the intact partner.

In the present investigation, several procedures known to increase ACTH secretion were applied to the intact partner. The change as well as the duration of the altered rate of secretion were determined by uniting the stressed animal with a hypophysectomized partner at various times after the application of the stress. Variations in the secretion of ACTH by the intact rat, after various procedures, are reflected by proportional changes in the adrenal ascorbic acid of the hypophysectomized partner.

EXPERIMENTAL METHODS

Male rats of the Sprague-Dawley strain, weighing 200–300 gm., were used in these experiments. The "test" animal (called the Donor), whose ACTH output was to be measured, was kept in a constant temperature room for at least one week prior to the experiment, and fed a diet of Purina chow and water, *ad libitum*. The Recipient "assay" animal was a hypophysectomized rat obtained from the Charles River Breeding Laboratories, Brookline, Mass. They were used 2-4 days post-operatively and were carefully examined, at the end of the experiment, for completeness of hypophysectomy. Only those results obtained from completely hypophysectomized Recipients are included in this report.

All animals were anesthetized with sodium pentobarbital (Nembutal), 5 mg./100 gm. body weight, given intraperitoneally at the time of the experiment. The operative

Received July 3, 1956.

[1] This investigation was supported by a research grant (PHS 256) from the Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service.

[2] The material presented herein was taken from a dissertation submitted to the Graduate School of Yale University by A. Brodish in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Physiology.

[3] National Science Foundation Postdoctoral Fellow. Part of this work was carried out during the tenure of the Porter Fellowship of the American Physiological Society.

cannulation procedure and apparatus for cross-circulation are described in a previous publication (1).

By means of the dorsal approach, one adrenal was removed from the hypophysectomized Recipient prior to the cross-circulation. This adrenal served as the control gland and was dissected free of adhering tissue, weighed on a Roller-Smith torsion balance, and placed in 6% trichloracetic acid for subsequent analysis of ascorbic acid concentration according to the method of Roe and Kuether (3).

Prior to the beginning of cross-circulation, both Donor and Recipient received intravenous injections of 1 mg. of heparin in a volume of 0.1 cc. At the end of the cross-transfusion of a specific quantity of blood (20 cc. transfer volume used in these experiments), the femoral blood vessels were tied off and the polyethylene cannulae were removed. Approximately 15–30 minutes after the end of the cross-circulation, the second or experimental adrenal gland of the Recipient was removed, cleaned, weighed, and prepared for subsequent ascorbic acid analysis.

Unilateral or bilateral adrenalectomies were performed via the dorsal approach. Bilaterally adrenalectomized animals were maintained on 0.9% sodium chloride throughout the experimental period. Surgical laparotomy was carried out by means of a midline incision through the abdominal wall and the gastro-intestinal tract was periodically manipulated during the course of the cross-circulation. Sciatic nerve stimulation was performed in the following manner: the sciatic nerve was exposed, dissected free of fascia, and the peripheral end cut. Wire electrodes were placed on the nerve and stimulation carried out by means of a square wave stimulator connected to a rotating drum which permitted the animal to receive the stimulation for 15 seconds of every minute. The parameters of stimulation were: frequency of 100/sec., pulse duration of 1 msec., and voltage of 7 volts.

RESULTS

The effect of various procedures on the discharge of ACTH in the intact Donor rat has been studied. The following procedures were employed: cannulation of femoral blood vessels, unilateral adrenalectomy, and bilateral adrenalectomy. Cannulation served as a mild stress whereas adrenalectomy represented a severe stress to the organism.

To follow the changes in the blood level of ACTH at any time subsequent to the application of the stress to the intact Donor, cross-circulation with a hypophysectomized Recipient was established and 20 cc. of Donor blood were exchanged and thereby assayed for ACTH content. In all experiments to be reported in this paper, 20 cc. were cross-circulated between Donor and Recipient animals since, in a previous publication (2), it was shown that this volume of blood from a normal Donor animal resulted in a 17.5% depletion of adrenal ascorbic acid—a value that is in the middle portion of the dose-response curve. In addition, radioactive isotope studies (Fe^{59}) have shown that twenty 1 cc. transfers result in equilibrium between the circulations of the cross-circulated partners (1). Furthermore, a different Donor animal was used at each time interval following the application of the specific experimental procedure under study. The altered concentration of ACTH in the blood of an animal was thereby determined, as far as possible, in response to the applied stress and uninfluenced by the concomitant effect of the cross-circulation procedure.

CANNULATION

A study was made of the effect of a mild stress, such as that associated with the cannulation of the femoral blood vessels, upon the release of ACTH from the anterior pituitary. The femoral artery and vein of the Donor were cannulated and the cannulae implanted under the skin of the animal. At varying periods of time, from 0.5 hour to 12 hours after cannulation, these Donors were cross-circulated with hypophysectomized Recipients. Table 1 indicates that a steady secretion of ACTH was maintained

TABLE 1. EFFECT OF CANNULATION ON DONOR'S ACTH OUTPUT AND ADRENAL ASCORBIC ACID RESPONSE

Time (hrs.)	Recipient			Donor		
	No. exp.	% A.A. change ±S.E.	P*	No. exp.	% A.A. change ±S.E.	P*
0 (control)	24	−17.5±1.70		134	0.0±1.06	
0.25				5	−20.5±4.88	<0.01
0.5				8	−29.3±2.54	<0.01
1.0	6	−15.6±3.99	>0.5	10	−31.0±5.72	<0.01
2.0	6	− 9.6±3.01	0.03	15	−42.1±3.66	<0.01
4.0	6	−11.7±2.82	0.08	10	−32.0±5.12	<0.01
6.0	6	−10.3±3.72	0.08	10	−30.2±3.57	<0.01
12.0	6	−11.4±2.21	0.036	5	0.0±2.24	>0.5

* Level of significance compared to controls

throughout a 12-hour period studied. This sustained rate of secretion was somewhat less than the initial control rate; nonetheless, these ACTH levels were easily detectable.

The adrenal ascorbic acid change in the intact rat, in response to the cannulation procedure, was determined in another group of animals. The results in Table 1 show that a progressive fall in the adrenal ascorbic acid occurred, reaching a maximum depletion in 2 hours. This was followed by a gradual return to initial levels 12 hours after cannulation. It is evident from Figure 1 that a lack of correlation exists between the level of ACTH in the blood and the changes in the adrenal ascorbic acid concentration of the intact Donor animal. Thus, the adrenal ascorbic acid changes in the intact animal indicate a varying rate of ACTH secretion despite the fact that the blood level analyses indicate a steady rate of ACTH secretion throughout this period. The true pattern of ACTH secretion cannot be accurately determined if one is limited to the use of the animal's own adrenals as the index of this secretion.

UNILATERAL ADRENALECTOMY

Elevated blood levels of ACTH have been demonstrated following the application of a severe stress such as unilateral adrenalectomy. An intact rat was unilaterally adrenalectomized, and the level of ACTH in the

blood at various times after the operation was determined by cross-transfusion with a hypophysectomized Recipient.

Table 2 shows that high blood levels of ACTH could only be demonstrated for one to two hours following the severe stress of unilateral adrenalectomy. Thereafter, diminishing blood levels were obtained which indeed reached non-detectable concentrations in 6 hours. However, resting levels were re-established in 12 to 24 hours and a new steady state was

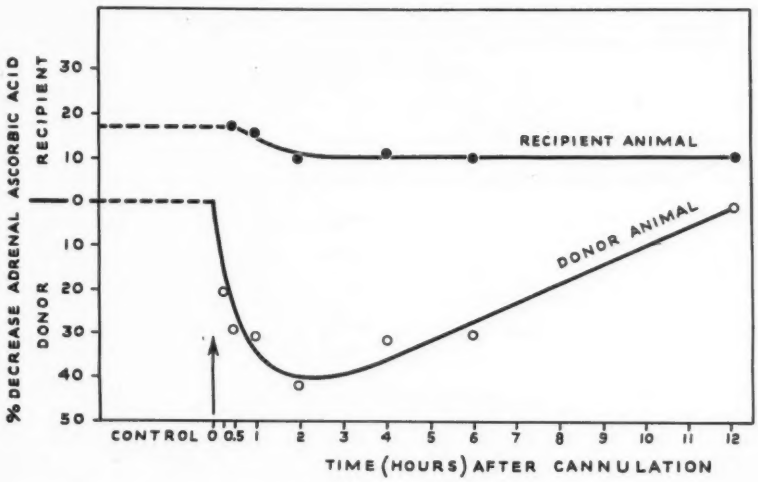


FIG. 1. Effect of cannulation on ACTH secretion and adrenal ascorbic acid response of normal rats. *Upper curve*: Blood level of ACTH in cannulated Donor animal estimated by the per cent adrenal ascorbic acid depletion of its hypophysectomized Recipient partner. *Lower curve*: Per cent change of adrenal ascorbic acid concentration within the adrenal of the cannulated Donor animal.

reached, at somewhat higher than normal values, one week after unilateral adrenalectomy.

A study was made of the effect of unilateral adrenalectomy on the ascorbic acid concentration of the remaining adrenal gland of normal rats. The excised gland (acting as the stress stimulus) served as the control gland in each case, whereas the second adrenal was removed at varying periods of time after extirpation of the control gland.

Table 2 demonstrates that after unilateral adrenalectomy in the rat, the fall in adrenal ascorbic acid begins with great rapidity and continues for about four hours. Thereafter, the concentration in the gland begins to increase, requiring 30 hours before the initial level is regained. Figure 2 shows that there is a lack of uniform correlation between the concentration of ACTH in the blood (as measured by the cross-circulation technique)

and the ascorbic acid concentration in the adrenal of the Donor animal at any given time after the stress. The rate of discharge of ACTH cannot be inferred merely from an analysis of the ascorbic acid depletion in the adrenal of the intact animal. Thus, although the ACTH level in the blood remains high for approximately one to two hours after removal of one adrenal, the adrenal ascorbic acid concentration of the Donor continues to decline for at least 4 hours. Although the initial high rate of ACTH

TABLE 2. EFFECT OF UNILATERAL ADRENALECTOMY ON DONOR'S ACTH OUTPUT AND ADRENAL ASCORBIC ACID RESPONSE

Time (hrs.)	Recipient			Donor		
	No. exp.	% A.A. change ±S.E.	P*	No. exp.	% A.A. change ±S.E.	P*
0 (control)	24	−17.5±1.70		6	+ 1.5±0.81	
0.25	6	−25.9±2.55	0.01	6	−20.7±0.86	<0.01
0.5				6	−26.4±2.32	<0.01
1.0	5	−25.4±1.22	<0.01	9	−34.5±2.28	<0.01
2.0				6	−44.9±3.56	<0.01
3.0	5	−11.4±2.59	0.056			
4.0				6	−47.0±5.87	<0.01
6.0	6	− 2.1±2.11	<0.01	6	−39.4±3.63	<0.01
9.0				6	−26.3±2.09	<0.01
12.0	5	−13.2±4.31	0.38	10	−20.6±3.21	<0.01
15.0				8	−15.2±2.80	<0.01
18.0				10	−13.1±3.14	<0.01
21.0				6	−10.4±3.80	0.01
24.0	5	−17.6±3.39	>0.5	6	−10.2±3.85	0.01
30.0				7	− 4.5±3.25	0.10
1 week	5	−23.1±1.56	0.02			
3 weeks	4	−23.3±5.25	0.28			
8 weeks	4	−21.1±2.41	0.24			

* Level of significance compared to controls.

discharge is not continued for more than a short period, this is nevertheless sufficient to cause a decline in the adrenal ascorbic acid for a period longer than ACTH can be detected in the blood. These results indicate that the adrenal ascorbic acid depletion in the intact rat does not necessarily reflect the blood content of ACTH at that particular time, for it can be seen (Fig. 2) that the adrenal ascorbic acid depletion is at a maximum at a time when the blood level of ACTH can be demonstrated to be approaching a minimum.

The disappearance of ACTH from the blood of animals 6 hours after removal of one adrenal was an unexpected finding. Sayers (4) has suggested that a high level of adrenal cortical steroids in the blood will depress ACTH release. It was conceivable that this might explain the observed pituitary inhibition, for it is presumed that the initial discharge of ACTH could raise the blood level of adrenal steroids by stimulation of the remaining adrenal to a point where depression of ACTH secretion occurs. However, this is not the case since the same phenomenon occurred after bilateral adrenalectomy.

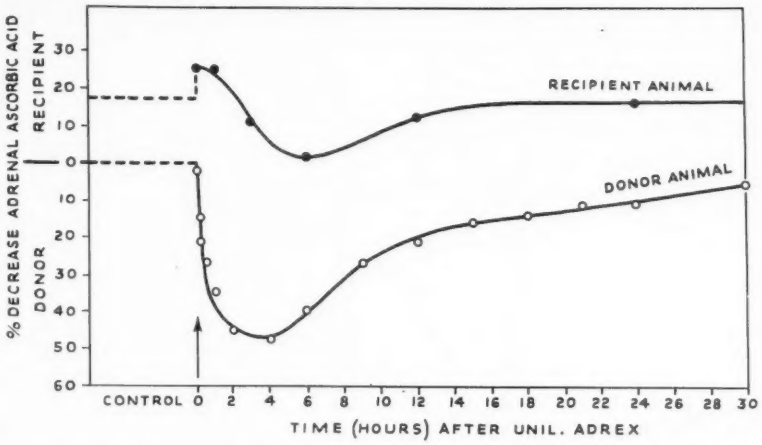


FIG. 2. Effect of unilateral adrenalectomy on ACTH secretion and adrenal ascorbic acid response of normal rats. *Upper curve*: Blood level of ACTH in unilaterally adrenalectomized Donor animal estimated by the per cent adrenal ascorbic acid depletion of its hypophysectomized Recipient partner. *Lower curve*: Per cent change of adrenal ascorbic acid concentration within the remaining adrenal of the unilaterally adrenalectomized Donor animal.

BILATERAL ADRENALECTOMY

In these experiments, both adrenals were extirpated within two minutes, a procedure that should have effectively prevented any substantial increase in the blood level of adrenal cortical steroids. Figure 4 shows that the same secretory pattern for ACTH was obtained following bilateral adrenalectomy as that which occurred after unilateral adrenalectomy (Fig. 3). However, high blood levels of ACTH were maintained for a shorter period of time in the former case than in the latter, since diminishing levels of ACTH were demonstrable one hour after bilateral adrenalectomy (*cf.* Table 3). Extremely low concentrations of blood ACTH were maintained over a longer period of time after bilateral adrenalectomy than was the case following unilateral adrenalectomy. Only after 24 hours were resting levels of ACTH re-established. Furthermore, these completely adrenalectomized animals reached the well-known steady state of supernormal ACTH levels two weeks after operation. This high rate of ACTH secretion was maintained for at least 4 weeks.

RESPONSE TO A SECOND STRESS

It has been shown that the high rate of ACTH secretion, following unilateral or bilateral adrenalectomy, was not maintained. Furthermore, the pituitary inhibition observed 6 hours after a severe stress was not due to a raised blood level of adrenal cortical hormones. Therefore a study was made to determine the ability of the anterior pituitary to secrete larger

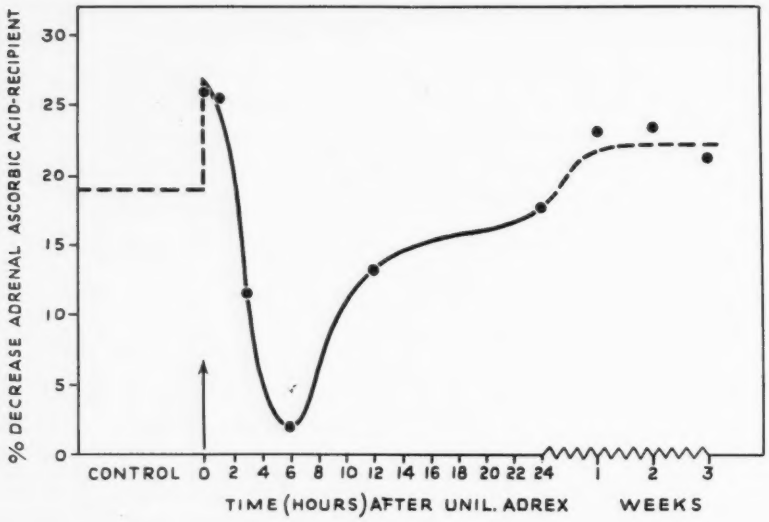


FIG. 3. Blood level of ACTH at various times following unilateral adrenalectomy of normal Donor rats. (Estimated by the per cent depletion in the adrenal ascorbic acid of its hypophysectomized Recipient partner.)

amounts of ACTH in response to a second stress applied 6 hours after the first.

Figure 5 demonstrates that 6 hours after unilateral adrenalectomy, a marked increase in the ACTH content of the blood was obtained after subjecting the animals to the following second stresses: Surgical laparotomy, removal of the second adrenal, and sciatic nerve stimulation. These results indicate that the disappearance of ACTH from the blood 6 hours after a severe stress, was probably not due to depletion of pituitary

TABLE 3. ACTH OUTPUT OF NORMAL RATS AT DIFFERENT TIMES AFTER BILATERAL ADRENALECTOMY

Time (hrs.)	No. exp.	Recipient	
		% A.A. change ±S.E.	P*
0 (control)	24	−17.5±1.70	
0.25	6	−25.9±2.19	<0.1
1.0	6	−18.8±3.68	>0.5
3.0	6	−10.8±1.94	0.015
6.0	6	− 3.3±2.34	<0.01
12.0	8	− 1.1±1.91	<0.01
24.0	7	−13.8±1.69	0.14
48.0	9	−14.5±2.97	0.38
72.0	7	−21.6±2.29	0.17
1 week	5	−24.8±2.82	0.037
2 weeks	5	−31.7±2.75	<0.01
3 weeks	5	−29.7±1.97	<0.01
4 weeks	5	−32.1±3.98	<0.01

* Level of significance compared to controls.

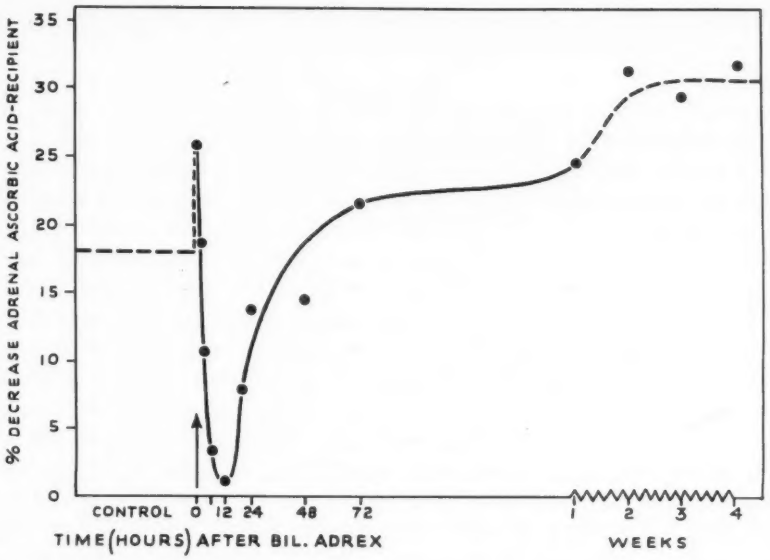


FIG. 4. Blood level of ACTH at various times following bilateral adrenalectomy of normal Donor rats. (Estimated by the per cent depletion in the adrenal ascorbic acid of its hypophysectomized Recipient partner.)

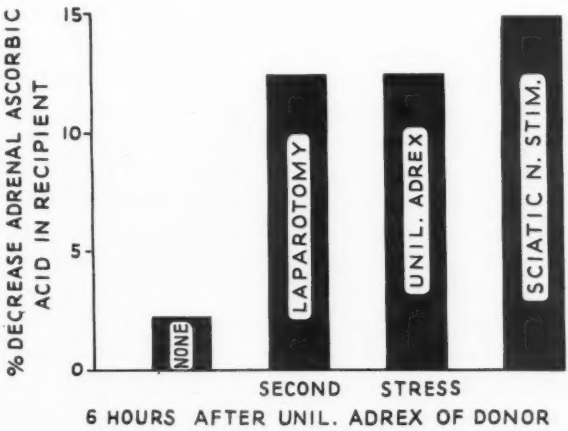


FIG. 5. Effect of application of a second stress on the level of ACTH in the blood of a Donor rat that had been unilaterally adrenalectomized 6 hours previously. (Estimated by the per cent depletion in the adrenal ascorbic acid of its hypophysectomized Recipient partner.)

stores of ACTH, for when a second stress was applied at this time, marked elevation of blood ACTH again occurred.

DISCUSSION

The capacity of a stressed animal to maintain a high rate of ACTH secretion has been studied. It has been shown that a high secretory rate can be maintained for only one to two hours following a severe stress. This is followed by a decline in ACTH release even though adequate pituitary stores remain. The virtual disappearance of ACTH from the body fluids 6 hours after a severe stress has not previously been reported by other investigators. Using the acid-acetone extraction procedure, Gemzell *et al.* (5) have observed an immediate and progressive increase in ACTH secretion following bilateral adrenalectomy of rats. Concomitantly, a progressive fall in pituitary ACTH content was noted during the first 24 hours following the removal of both adrenals. These investigators obtained their blood samples from the vena cava of rats anesthetized with ether at time intervals comparable to those of the present investigation—i.e. 10 min., 1 hour, 3 hours, 6 hours, 10 hours, 16 hours, 20 hours, etc. These investigators (confirmed in the present study), demonstrated that 3 weeks after bilateral adrenalectomy a new steady state is reached characterized by a markedly greater blood ACTH titer. The reason for the discrepancy (in the early stages) between the results obtained by these workers and those of the present investigation is not apparent. However, it may be due to the different methods used for estimation of blood ACTH and/or the anesthetic agent employed.

The data of this investigation have shown that a moderate rate of ACTH secretion can be sustained for a long period of time following a mild stress such as cannulation of the femoral blood vessels, whereas a high rate of ACTH output after a severe stress of unilateral or bilateral adrenalectomy is not maintained. Indeed, practically no ACTH can be detected in the blood 6 hours after a severe stress. From the studies on bilaterally adrenalectomized animals, it is probable that this pituitary inhibition is not due to an increased level of adrenal cortical hormone. Neither is this phenomenon the result of exhaustion of pituitary ACTH stores, since the pituitary is capable of releasing additional hormone upon further stimulation at this time. Additional evidence of this phenomenon has been reported by Sayers and Burke (6). These investigators have observed the inability of bilaterally adrenalectomized rats to maintain a high rate of ACTH secretion following administration of ether anesthesia. A progressive decline in the level of circulating ACTH was observed even though pituitary ACTH content remained unchanged.

Adrenalectomized animals can maintain a high secretory rate of ACTH output two weeks after operation, whereas, immediately after removal of both adrenal glands, they do not secrete large quantities of ACTH for

more than a two-hour period. This suggests that some new mechanism, not present initially, may have been brought into play to permit these animals to maintain such high blood levels of ACTH.

The results of this study demonstrate the existence of a biphasic pattern of ACTH secretion (Fig. 3 and 4). A rapid, increased secretion of ACTH, in response to stress, has been demonstrated. This rapid, short-acting ACTH secretory phase is probably under neural regulation and is not maintained for more than two hours. Following this initial phase, a second more prolonged phase of ACTH secretion has been shown to ensue. The second, slowly developing, prolonged secretory phase may be indicative of the intervention of humoral regulation by adrenal cortical hormones. It remains for further work to substantiate this hypothesis. However, indirect evidence is available which may be interpreted in accordance with this hypothesis. Experiments of Fry (7), using rats with high spinal cord section, indicate the existence of a dual regulatory mechanism for ACTH secretion in response to stress. When the spinal cord of the rat is transected at the level of the second to third thoracic vertebrae, no ascorbic acid depletion is evident when the adrenals are taken one hour apart. This has also been reported by Weisz and co-workers (8). However, if the delay between the adrenal gland extirpation is extended to four hours, a significant fall in adrenal ascorbic acid is observed. This indicates that in animals with this specific neurological lesion, the rapid pituitary release is prevented, whereas a slowly developing delayed response mechanism is still in operation. Other investigagors have also obtained evidence of the existence of a dual mechanism of ACTH secretion (9, 10, 11, 12).

SUMMARY

The change as well as the duration of the altered rate of ACTH secretion, in response to stress, were determined by means of a cross-circulation technique.

It has been demonstrated that the high rate of ACTH secretion, following the application of a severe stress such as unilateral or bilateral adrenalectomy, can be maintained for approximately two hours, after which time the blood ACTH level gradually diminishes and is not detectable in 6 hours. However, after a milder stress, such as that associated with the cannulation of the femoral blood blood vessels prior to cross-circulation, a steady moderate rate of ACTH secretion can be maintained throughout a 12-hour period studied.

The disappearance of ACTH from the blood 6 hours after a severe stress is not due to increased blood level of adrenal cortical hormones, since it also occurs after bilateral adrenalectomy. Neither is this phenomenon due to exhaustion of pituitary ACTH, for if a second stress is applied at this time, marked elevation of blood ACTH occurs.

A biphasic pattern of ACTH secretion has been demonstrated following

the application of a severe stress. The existence of a dual mechanism regulating ACTH secretion is discussed.

Acknowledgment

Heparin supplied through the kindness of Dr. K. W. Thompson, of Organon, Inc., Orange, New Jersey.

REFERENCES

1. Brodish, A. and C. N. H. Long: *Yale J. Biol. Med.* **28**: 644. 1956.
2. Brodish, A. and C. N. H. Long: *Yale J. Biol. Med.* **28**: 650. 1956.
3. Roe, J. H. and C. A. Kuether: *J. Biol. chem.* **147**: 399. 1943.
4. Sayers, G. and M. Sayers: *Rec. Prog. Horm. Res.* **2**: 81. 1948.
5. Gemzell, C. A., D. C. Van Dyke, C. A. Tobias and H. M. Evans: *Endocrinology* **49**: 325. 1951.
6. Sayers, G. and R. Burke: *Fed. Proc.* **14**: 131. 1955.
7. Fry, E. G.: Unpublished observations.
8. Weisz, P., E. Glaz, S. Z. Vaseszky and T. Gati: *Acta med. Acad. Scient. Hung.* **4**: 201. 1953.
9. McDermott, W. V., E. G. Fry, J. R. Brobeck and C. N. H. Long: *Yale J. Biol. Med.* **23**: 52. 1950.
10. Fortier, C.: *Endocrinology* **49**: 782. 1951.
11. Sawyer, C. H. and G. R. Parkerson, Jr.: *Endocrinology* **52**: 346. 1953.
12. Anand, B. K., P. Raghunath, S. Dua and S. Mohindra: *Ind. J. Med. Res.* **42**: 231. 1954.

ACTH CONTENT OF RAT PITUITARY GLANDS

MARION K. BIRMINGHAM, ERIKA KURLENTS, G. J. ROCHEFORT, M. SAFFRAN, AND A. V. SCHALLY

Allan Memorial Institute of Psychiatry, McGill University, Montreal, Quebec, Canada

IN STUDIES of changes in the ACTH content of rat pituitary glands, the resting value and its usual range must be firmly established. There are anly a few reports of such values. For example, Burns, Merkin, Sayers, ond Sayers (1) found about 100 mU. of ACTH per rat pituitary, and Sydnor and Sayers (2) reported a value of 70 mU. per gland. More recently, Saffran and Schally (3), using a different method of assay, found values ranging about 300–400 mU. per 5–6 mg. rat gland. The discrepancy could result from different methods of extraction and/or different bioassay procedures. In the course of experiments designed to detect the source of the disagreement, we found that the extraction procedure is probably the major variable and that it is possible to extract more than 1000 mU. from each rat pituitary gland—10 times as much as the values first reported by Burns *et al.*!

METHODS

HCl-extraction. The anterior lobe of a rat pituitary was removed as described by Saffran and Schally (3), weighed rapidly on a micro-torsion balance, and placed into a small tube containing 100 μl. of 0.01N HCl. The tissue was ground as finely as possible with a glass rod and a few grains of sand. The HCl extract was stored in the refrigerator and was assayed, after suitable dilution with Krebs-Ringer-bicarbonate-glucose medium, by the *in vitro* method of Saffran and Schally (4) against U.S.P. standard ACTH, or other preparations that had been standardized with the U.S.P. standard with this assay.

Glacial acetic acid extraction. The gland was weighed and ground as above in 100 μl. of glacial acetic acid and heated, with stirring, at 70° for 30 minutes. The rod was rinsed into the tube with 100 μl. of glacial acetic acid and the stoppered extract was stored in the refrigerator. Before assay, the extract was diluted to 2 ml. with water and was neutralized with a molar solution of $NaHCO_3$. An aliquot was then diluted with medium for assay.

Modified glacial acetic acid method. The weighed gland was ground with sand with 500 μl. of glacial acetic acid. A further 500 μl. of acetic acid was added and the tube was heated, with stirring, in a water bath kept at 85° for 30 minutes. Care was taken to exclude condensed water from the tube. The tube was removed from the bath and the rod was rinsed into the tube with 500 μl. of acetic acid. The tube was stoppered with parafilm and was stored in the deepfreeze. The day before the assay the tube was thawed and placed into a vacuum desiccator over NaOH pellets at room temperature. In the morning, the dry residue was extracted with 800 μl. of 0.01N HCl at room temperature for 2 hours and a 100 μl. aliquot was diluted with medium for assay.

Received July 5, 1956.

TABLE 1. ACTH ACTIVITY IN DILUTE HCl EXTRACT OF RAT PITUITARY GLANDS

Duration of storage (days)	ACTH per mg. fresh tissue (mU.)	95% confidence limits (mU.)	Index of precision (λ)
2	32	15– 68	0.16
2	42	14–132	0.11
2	44	35– 56	0.06
2	68*	39–119	0.11
3	24[1]	7– 79	0.24
5	25[2]	13– 49	0.13
5	45	24– 84	0.12
5	45	22– 92	0.12
5	62	28–138	0.18
5	64	20–203	0.22
5	75	63– 89	0.04
6	62	32–119	0.14
6	82	52–129	0.10
10	102	61–170	0.11
10	148*	73–302	0.13

Mean, excluding 10 day old extracts, 52 mU.

* Same extract.

[1,2] Half pituitaries. The remaining halves were extracted with glacial acetic acid; see Table 2.

RESULTS

Table 1 lists the ACTH content of rat pituitary glands determined by the dilute HCl extraction; Table 2, by the glacial acetic acid extraction; Table 3, by the modified glacial acetic acid procedure. The mean value obtained with dilute HCl, 52 mU. per mg. (about 300 mU. per gland), is far lower than the amounts found with glacial acetic acid, 145 mU. per mg. (about 800 mU. per gland), and with the modified glacial acetic acid method, 241 mU. per mg. (about 1300 mU. per gland). The values found with the dilute HCl extraction tended to increase with time of storage of the extract in the refrigerator before assay.

DISCUSSION

Acetic acid extraction yields about 5 times as much ACTH activity as extraction with dilute HCl, in spite of claims that HCl extraction is

TABLE 2. ACTH ACTIVITY IN GLACIAL ACETIC ACID EXTRACTS OF RAT PITUITARIES

ACTH per mg. fresh tissue (mU.)	95% confidence limits (mU.)	Index of precision (λ)
116	48–281	0.18
118[2]	82–169	0.08
119	66–120	0.11
147	71–302	0.11
147	72–300	0.15
224[1]	77–650	0.19

Mean, 145 mU.

[1,2] Half pituitaries. The remaining halves were extracted with dilute HCl; see Table 1.

quantitative (1). In addition, the amount of ACTH extracted by the HCl seems to depend upon the time of storage of the extract. The development of the aceticacid-oxycellulose method for the preparation of ACTH (5) has multiplied the availability of ACTH, implying an increased yield from the tissue, although there are no reliable figures to be found in the literature.

Our finding of above one thousand mU. of ACTH in a single rat pituitary gland makes necessary the reevaluation of experiments in which the pituitary content of ACTH is examined after stress. The stores of ACTH are so large that only a relatively great change would be detected by the

TABLE 3. EXTRACTION OF ACTH BY THE MODIFIED GLACIAL ACETIC METHOD

mU./mg.	95% limits	λ
170	80–360	0.16
218	141–338	0.10
233	106–513	0.16
250	147–428	0.11
334	154–724	0.15
Mean, 241 mu.		

existing methods of assay. This would account for the failure to find significant ACTH depletion after stress and for the relatively slight change after adrenalectomy (2).

Although most of the discrepancy between older values and the values in this paper can be attributed to different extraction procedures, there remains the possibility that the two assay methods used contribute to the difference because HCl-extraction yields, with the *in vitro* assay, about 300 mU. per gland, and only 70–100 mU. with the adrenal ascorbic acid depletion assay (6). The ascorbic depletion assay has proven to be reliable and extremely useful in recent years, but its basis, the relationship between adrenal ascorbic acid and the stimulation of corticosteroidogenesis by ACTH, is still not understood. On the other hand, the *in vitro* bioassay is based upon the direct determination of the stimulation by ACTH of the formation of corticoids. Although the assay of most ACTH preparations by both methods yields almost identical results (4, 7), the ascorbic acid-depleting and corticoid-stimulating properties of ACTH may not be identical under all circumstances. The native ACTH in the pituitary may have more corticoid-stimulating activity than ascorbic acid depleting activity.

SUMMARY

A glacial acetic acid extract of a single rat pituitary gland contains approximately 1.3 units of ACTH, as determined by the *in vitro* assay of Saffran and Schally. This figure is 10–20 times higher than the amounts

found by dilute HCl extraction and the adrenal ascorbic acid depletion assay of Sayers, Sayers and Woodbury. Most of the discrepancy can be attributed to the superior extraction properties of glacial acetic acid, but the possibility remains that the native ACTH in the pituitary is more potent than is indicated by its adrenal ascorbic acid depleting activity.

Acknowledgments

This work was supported by a Federal-Provincial Mental Health Grant, and by grants from the Foundations' Fund for Research in Psychiatry and the 33rd Scottish Rite Committee for Research in Dementia Praecox, to Dr. R. A. Cleghorn. Equipment to facilitate the assay of ACTH was obtained through the kindness of Charles E. Frosst and Company Limited, Montreal.

REFERENCES

1. Burns, T. W., M. Merkin, M. A. Sayers and G. Sayers: *Endocrinology* **44**: 439. 1949.
2. Sydnor, K. L. and G. Sayers: *Endocrinology* **55**: 621. 1954.
3. Saffran, M. and A. V. Schally: *Can. J. Biochem. Physiol.* **33**: 408. 1955.
4. Saffran, M. and A. V. Schally: *Endocrinology* **56**: 532. 1955.
5. Astwood, E. B., M. S. Raben and R. W. Payne: *Recent Progress in Hormone Research* **7**: 1. Academic Press, Inc., New York. 1952.
6. Sayers, M. A., G. Sayers and L. A. Woodbury: *Endocrinology* **42**: 379. 1948.
7. McKerns, K. W. and E. Nordstrand: *Can. J. Biochem. Physiol.* **33**: 681. 1955.

GROWTH HORMONE CONTENT IN HUMAN PITUITARIES[1]

CARL A. GEMZELL AND FRANK HEIJKENSKJÖLD

Department of Obstetrics and Gynecology, Karolinska Sjukhuset and Konung Gustaf V's Forskningsinstitut, Stockholm

GROWTH hormone (GH) has been isolated from ox pituitaries (1, 2), pig pituitaries (3) and from horse, sheep and fish pituitaries (4). The hormone isolated from different species varies in physical and chemical properties. Thus, the difference in isoelectric points among the various GH preparations indicates differences in composition and possibly also in structure. Furthermore, the biological activity of the different GH preparations varies from species to species. A preparation may be inactive when tested in one species but highly active when tested in another species.

A rich literature has accumulated on the effects of GH in animals, not only on growth but on a wide variety of metabolic processes as well. Evidence for its action in man is, on the other hand, meager and inconclusive. GH preparations with equal growth promoting properties in animals are not equally or consistently effective in achieving metabolic changes in man. Some of these discrepancies may be due to inadequate dosage, antihormone formation or contamination of GH with other trophic factors with activities which may mask or counteract those of GH. The discrepancy may also be due to the specificity of the GH preparations isolated from different sources. Thus, GH from animal pituitaries may be inactive in man while GH from human pituitaries may show activity in man.

This paper is concerned with the isolation of GH from human pituitaries collected at autopsy.

MATERIAL AND METHODS

The pituitaries were collected at autopsy one to three days after death. No attempt at selection of patients was made. It should be emphasized that none of the patients can be considered "normal" in the sense that they met a sudden accidental death; all had been subjected to the stress of a fatal illness for a more or less prolonged time.

The whole pituitary was immediately frozen, ground with solid carbon dioxide, treated with cold acetone and lyophilized. As it may be suggested that manipulation of the gland before it is frozen may destroy hormone activity, no attempt was made to separate the anterior lobe of the pituitary from the posterior lobe. The acetone powder was then treated according to the method described by *Li* (5) for isolation of GH from ox pituitaries. The procedure of Li was followed to fraction A. The obtained yield was assayed for GH, adrenocorticotrophic hormone (ACTH), thyrotrophic hormone (TSH) and gonadotrophins.

Received July 18, 1956.

[1] Supported by a grant from Statens medicinska forskningsråd.

The GH activity of the pituitary preparations was assayed by the tibia test as described by Evans, Simpson, Marx and Kibrick (6). A GH preparation prepared by Dr. C. H. Li from ox pituitaries was used as a reference standard. In doses of 20 to 100 μg. this preparation gave a straight line relationship between the epiphyseal-cartilage readings in micra and the logarithm of the dose (Fig. 1). This line had an average slope of 59.7 and the assay an index of precision (λ) of 0.39.

As an assay method for ACTH the adrenal ascorbic acid depletion method of Sayers, Sayers and Woodbury (7) was employed. The assay animals were male albino rats weighing around 120 gm. The rats were hypophysectomized 48 hours before the assay. A stand-

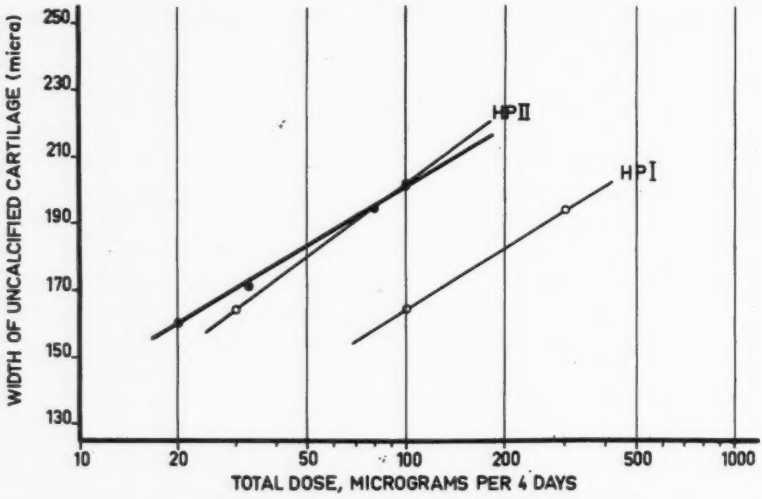


FIG. 1. Dose-response curves of the tibial cartilage response to a standard GH preparation of Li (thick line) and HP I and HP II.

ard dose-response curve was prepared from an U.S.P.-preparation. The curve had an average slope of 81.0 and the assay an index of precision (λ) of 0.33.

A combined assay was also performed in hypophysectomized male rats. The samples were injected intraperitoneally once a day during a two weeks' period starting the day after hypophysectomy. The weight of the animals, the thyroids, the adrenals, the seminal vesicles and the testis were recorded. No data from incompletely hypophysectomized animals was included.

The content of thyrotrophic hormone was estimated by the assay of Rowlands and Parkes (8).

RESULTS

Fractionation of two batches of human pituitaries was performed. In the first batch 12 pituitaries were treated and fraction A gave a yield of 176 mg. (HP I). In the second batch 20 pituitaries were treated and fraction A gave a yield of 130 mg. (HP II). The two end fractions were easily soluble in saline at pH 4.0 (Table 1).

HP I and HP II were assayed for GH by the tibia test. A symmetrical

TABLE 1. YIELD OF FRACTION A FROM TWO BATCHES OF HUMAN PITUITARIES

Preparation	No. of human pituitaries	Total wet weight of pituitaries	Acetone powder	Yield (Fraction A)
		gm.	gm.	mg.
HP I	12	8.5	1.68	176
HP II	20	16.0	2.35	130

four-point assay with two dose levels of the standard preparation and the unknown preparation and with an almost equal spacing of log doses was performed. HP I gave in a dose of 100 μg. an average width of the tibial epiphyseal cartilage plate of 165 micra and in a dose of 300 μg. a width of 194 micra. The dose-response curve of HP I showed a slope of 60.1 as compared to the slope of 62.9 for the standard preparation. The percentage potency of HP I in comparison to the GH standard was 25.1. Thus, in HP I an equivalent of 44.1 mg. of GH was found or 3.7 mg. per pituitary (Fig. 1, Tables 2 and 3).

HP II gave in a dose of 30 μg. an average cartilage width of 164 micra and in a dose of 100 μg. a width of 202 micra. The dose-response curve showed a slope of 72.9 against 56.5 for the standard preparation. The percentage potency of HP II was 92.3. Thus, in HP II an equivalent of

TABLE 2. GH ASSAY OF HP I AND HP II

Preparation	Total dose	No. of assay rats	Average width of tibial epiphyseal cartilage plate ± stand. error	Variance	Slope	Average slope (b)	Standard deviation (s)	Degrees of freedom	$\lambda = \frac{s}{b}$
	μg.		micra						
HP I	100	8	165 ± 3.4	94	60.1 ± 20.2				
HP I	300	9	194 ± 8.5	653		61.1 ± 19.8	24.1	22	0.39
Li	33	4	172 ± 14.3	816	62.9 ± 43.7				
Li	100	5	202 ± 14.9	1108					
HP II	30	10	164 ± 8.2	671	72.9 ± 22.6				
HP II	100	13	203 ± 8.2	881		67.7 ± 17.3	26.1	27	0.39
Li	20	4	160 ± 9.4	351	56.5 ± 20.2				
Li	80	4	194 ± 7.8	242					

TABLE 3. GH POTENCY OF HP I AND HP II (THE STANDARD PREPARATION OF Li = 100%)

Preparation	Average width of tibial epiphyseal cartilage plate		Average log dose		Log relative potency	Relative potency
	Test	Standard	Test	Standard		
	micra	micra				
HP I	180.2	188.3	2.253	1.786	−0.600 ± 0.169	0.251 ± 0.098
HP II	186.0	176.8	1.773	1.602	−0.035 ± 0.162	0.923 ± 0.344

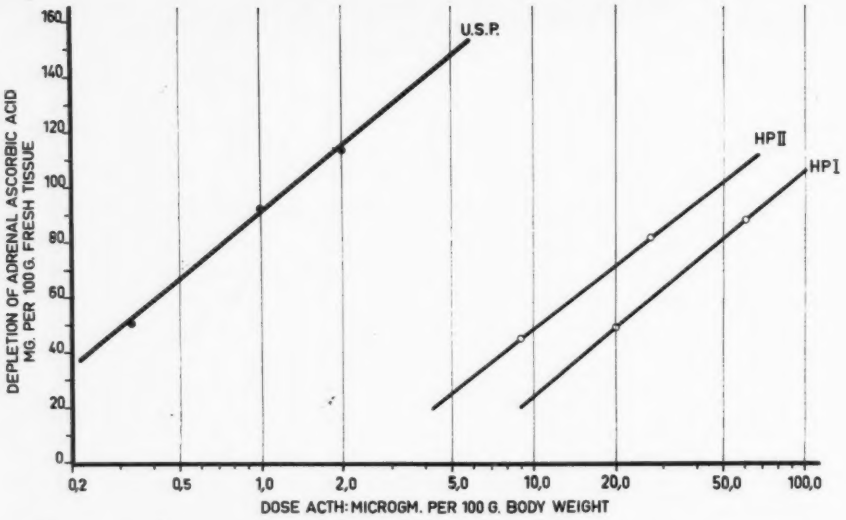


FIG. 2. Dose-response curves of the adrenal ascorbic acid depletion of an U.S.P. preparation and HP I and HP II.

120 mg. of GH was found or 6.0 mg. per pituitary (Fig. 1, Tables 2 and 3).

HP I and HP II were also tested in the high dose of 1000 μg. The average response (198 and 243 micra respectively) was lower than would be expected from the results of the smaller doses. These results seemed to indicate that the two preparations were contaminated with substances that counteracted the effect of GH. This substance may be ACTH which is known to inhibit the effect of GH on the tibia epiphysis (9).

HP I and HP II were therefore assayed for ACTH by the ascorbic acid depletion method of Sayers, Sayers and Woodbury (7). A four-point assay with three doses of U.S.P. and two doses of the unknown (HP I & HP II) was performed (Fig. 2, Table 4). HP I in a dose of 20 μg. gave an average

TABLE 4. ACTH ASSAY OF HP I AND HP II

Preparation	Total dose	No. of assay rats	Average ascorbic acid depletion ± stand. error	Variance	Slope	Average slope	Standard deviation	Degrees of freedom	$\lambda = \frac{s}{b}$
	μg.		mg./100 g. tissue						
HP I	20	8	48.5± 8.3	557	81.9±31.9				
HP I	60	7	87.6±13.2	1219					
HP II	9	6	43.8± 5.8	202	77.5±26.6				
HP II	27	11	80.8± 8.7	834		80.3±13.9	26.4	44	0.33
U.S.P.	0.33	6	50.0± 8.0	384					
U.S.P.	1.0	6	92.2±14.1	1201	81.0±18.5				
U.S.P.	2.0	6	112.8± 8.6	444					

TABLE 5. ACTH POTENCY OF HP I AND HP II (U.S.P. = 100%)

Preparation	Average ascorbic acid depletion		Average log dose		Log relative potency	Relative potency
	Test	Standard	Test	Standard		
	mg./100 g. tissue	mg./100 g. tissue				
HP I	66.7	85.0	1.524	−0.090	−1.842 ± 0.122	0.0144 ± 0.0040
HP II	67.8	85.0	1.263	−0.090	−1.568 ± 0.117	0.0270 ± 0.0073

ascorbic acid depletion of 48.5 mg./100 gm. tissue and in a dose of 60 μg. an average depletion of 87.6 mg./100 gm. tissue. The slope of the dose-response curve was 81.9 which agreed well with the slope of the U.S.P. of 81.0. The percentage potency of HP I was 1.4 (Table 5). Thus, 14 μg. of ACTH was found in each mg. of HP I.

HP II was assayed in doses of 9 and 27 μg. and gave an average depletion of 43.8 mg./100 gm. tissue and 80.8 mg./100 gm. tissue respectively (Fig. 2, Table 4). The dose-response curve showed a slope of 77.5 and the percentage potency of HP II in comparison with U.S.P. was 2.7 (Table 5). Thus, 27 μg. of ACTH was found in each mg. of HP II.

One mg. of HP II was injected intraperitoneally into hypophysectomized rats daily for 14 days (Table 6). At autopsy the injected rats had increased 3.1 gm. in weight per day. The thyroids showed only a slight weight increase. The adrenals increased 0.65 mg. per day. The seminal vesicles increased about 20 mg. per day and the testis 45 mg. per day. These effects of HP II in hypophysectomized rats seemed to indicate that the preparation was contaminated not only by ACTH which has been proved before but also by gonadotrophins. The gonadotrophic assay was not further differentiated into special assays for follicle stimulating hormone (FSH) and luteinizing hormone (LH) but both these trophic hormones seemed to be present. Neither was the result of this assay compared with the effect of known amounts of gonadotrophins in hypophysectomized rats. Consequently, no quantitative estimations of the gonadotrophins were performed.

TABLE 6. A COMBINED ASSAY FOR GH, TSH, ACTH AND GONADOTROPHINS IN HYPOPHYSECTOMIZED MALE RATS. 1 MG. OF HP II INJECTED INTRAPERITONEALLY ONCE A DAY FOR 14 DAYS

	No. of rats	Body wt.	Thyroids	Adrenals	Ves. seminal.	Testis
		g.	mg.	mg.	mg.	mg.
Normal	4	208 ± 1.3	17 ± 1.5	41 ± 4.5	290 ± 36.0	2310 ± 142
Hypophysectomized	3	114 ± 6.4	10 ± 1.8	10 ± 1.0	15 ± 0.4	359 ± 19
Hypophysectomized +1 mg. of HP II/day in 14 days	4	158 ± 1.9	12 ± 1.4	19 ± 1.1	288 ± 25.0	980 ± 73

HP I and HP II were also assayed for TSH. To each assay guinea pig an amount of 3.75 mg. of HP I or HP II was administered. This dose showed no TSH activity. Due to the sensitivity of the assay method, it could be stated, therefore, that the contamination of TSH in these preparations was less than 0.167 U.S.P.-units per mg.

Due to the lack of material no investigations on physicochemical properties of the two isolated fractions were performed. However, paper electrophoresis showed that the two fractions migrated with the same speed as the pure GH preparation of Li and showed a single component. A further investigation on the physicochemical properties of human pituitary GH will be published later.

DISCUSSION

Few accounts have been found in the literature pertaining to the content of trophic hormones in the human pituitary. Mostly the content of gonadotrophins have been investigated (10). The presence of other trophic hormones as TSH and ACTH have been proved by Taylor (11), Burt and Velardo (12) and Hewett, Cruickshank and Currie (13). Burt and Velardo (12) injected frozen, homogenized human pituitaries into hypophysectomized rats and showed an increase in the weight of adrenals, thyroids and gonads. Taylor (11) showed ACTH activity in lyophilized human pituitaries and Hewett *et al.* (13) treated an acetone powder of human pituitaries with acid acetone and received a fraction that was highly active when tested by the ascorbic acid depletion method of Sayers *et al.* (7).

This study has shown that human pituitaries contain GH in an amount of 3.7–6.0 mg. per gland as compared to a standard GH preparation of Li. As a fresh human pituitary weighed about 1 gm. the amount of isolated GH seemed to correspond fairly well with the amount of GH isolated from fresh ox pituitaries. The inactivation of GH after death, therefore, can hardly proceed at a high rate.

The pituitaries were chosen at random and no correlation could be observed between the content of GH in the pituitaries and the age and sex of the patients. However, as the majority of the collected pituitaries originated from old patients it might be assumed that GH can be extracted not only from the pituitaries of growing individuals but also from the pituitaries of old people.

Bioassays for trophic hormone in the anterior pituitary measure only stored material and give no information about the rates of production and release of this hormone. There is evidence, however, linking amount of stored hormone to the rate of production and secretion of the hormone (14). Following adrenalectomy in rats, elevated plasma levels of ACTH were found together with an increased amount of stored ACTH in the pituitary.

SUMMARY

Human pituitaries collected at autopsy, one to three days after death were treated according to the method described by Li for isolation of GH from ox pituitaries. The fraction obtained was contaminated with ACTH (1.4–2.7%), gonadotrophins (FSH and LH) and probably also with posterior lobes hormones (not tested). No TSH was found.

The content of GH in each pituitary was 3.7–6.0 mg. as compared to a standard GH preparation, obtained by Dr. C. H. Li.

Acknowledgments

The authors want to thank Dr. C. H. Li, Berkeley, for the generous gift of a pure growth hormone preparation. We are also greatly indebted to Mrs. Lisa Gemzell and Mrs. Mona Jäderholm for expert technical assistance.

REFERENCES

1. Li, C. H. Evans, H. M. and Simpson, M. E.: *J. Biol. Chem.* **159**: 353. 1945.
2. Wilhelmi, A. E., J. B. Fishman, and J. A. Russel: *J. Biol. Chem.* **176**: 735. 1948.
3. Raben, M. S. and V. W. Westermeyer: *Proc. Soc. Exp. Biol. Med.* **78**: 550. 1951.
4. Wilhelmi, A. E.: Hypophyseal Growth Hormone, McGraw-Hill Book Co. 59. 1954.
5. Li, C. H.: *J. Biol. Chem.* **211**: 555. 1954.
6. Evans, H. M., M. E. Simpson, W. Marx, and E. Kibrick: *Endocrinology* **32**: 13. 1943.
7. Sayers, M. A., G. Sayers, and L. A. Woodbury: *Endocrinology* **42**: 379. 1948.
8. Rowlands, I. W. and A. S. Parkes: *Biochem. J.* **28**: 1829. 1934.
9. Geschwind, I. I. and C. H. Li: *J. Clin. Endocrinol. & Metab.* **12**: 937. 1952.
10. Bahn, R. C., N. Lorenz, W. A. Bennet and A. Albert: *Endocrinology* **52**: 135. 1953.
11. Taylor, N. R. W.: *J. Endocrinol.* **9**: 334. 1953.
12. Burt, A. S. and J. T. Velardo: *J. Clin. Endocrinol. & Metab.* **14**: 979. 1954.
13. Hewett, C. L., B. Cruickshank, and A. R. Currie: *Nature* **174**: 745. 1954.
14. Gemzell, C. A., D. C. VanDyke, C. A. Tobias, and H. M. Evans: *Endocrinology* **49**: 325. 1951.

THE INFLUENCE OF ESTROGEN UPON GOITER INDUCTION IN ADULT AND IMMATURE RATS

B. A. ESKIN[1] AND E. M. BOGDANOVE

Department of Anatomy, Albany Medical College of Union University, Albany, New York

INTRODUCTION

THERE have been many conflicting reports as to the way in which the thyroid glands of experimental animals are affected, if at all, by the administration of estrogenic hormones. The subject has been carefully reviewed by Noach (1, 2). In adult, propylthiouracil[2]-treated rats, we have found that the concurrent administration of an estrogen, α-estradiol benzoate[2], reduces the hyperplastic response of the thyroid gland to the goitrogen. The present report deals with this inhibitory phenomenon, and with the effects thereon of age, sex, strain and variations in drug dosage.

MATERIALS AND METHODS

A total of 281 male and female rats (from 20 days to 10 months-in-age) of the Vanderbilt, Sprague-Dawley, and Wistar strains was used in this study. Batches of animals of the same strain, sex, age and approximate weight were divided into the various experimental and control groups for each run, and kept under identical conditions, except for the variations in drug administration. They were given a standard diet and tap water *ad libitum*. The experiments were run at intervals over a three year period, during which time three different brands of dog pellets were used as the standard diet for rats in this laboratory.

Propylthiouracil (PTU) was administered either as a 5 mg.% solution in the drinking water, or injected subcutaneously once a day as 0.5 ml. of an aqueous suspension (with an equal amount of gum acacia) containing the desired dose. α-estradiol benzoate (EB) was injected daily, subcutaneously, as either 0.1 or .2 ml. of a sesame oil solution. When both drugs were injected, they were given concomitantly but at different sites. A few of the controls were injected with sesame oil alone, but most were uninjected. A few rats received EB but not PTU. Since the thyroid weights of rats given either sesame oil alone or with the estrogen did not differ from those of the untreated rats, these data have been pooled as control data. The treatment period was either 20 or 21 days.

The rats were sacrificed on the day following the last injection. The thyroid glands were trimmed, and weighed on a torsion balance to the nearest 0.5 mg. The values were converted to relative weight (mg./100 gm. body wt.). Representative glands from each group of rats were fixed in Zenker-Formol and examined histologically.

Received July 20, 1956.

[1] Present address: Department of Gynaecology, Hospital of Woman's Medical College, Philadelphia 29, Pa.

[2] We are indebted to Drs. Edward Henderson and Norman L. Heminway of the Schering Corporation for generous donations of α-estradiol benzoate, and to Dr. G. H. Schneller of the Calco Chemical Division, American Cyanamid Company for kindly supplying the propylthiouracil used in this study.

Where the size of the pooled samples permitted, the data were analyzed statistically by the method of Student's *t*. Data from groups too small for such treatment, which serve to extend the statistically evaluated findings, have also been included in this report. For clarity, the specific dosages and number of animals used in each group have been indicated in the section on results.

RESULTS

Influence of age on EB effect on goiter development. The mean thyroid weight of 23 adult female rats given PTU (5 mg.%) *per os* for 20 days, was 20.9 ± .83 mg./100 gm., as shown in Fig. 1. Similar rats given PTU by

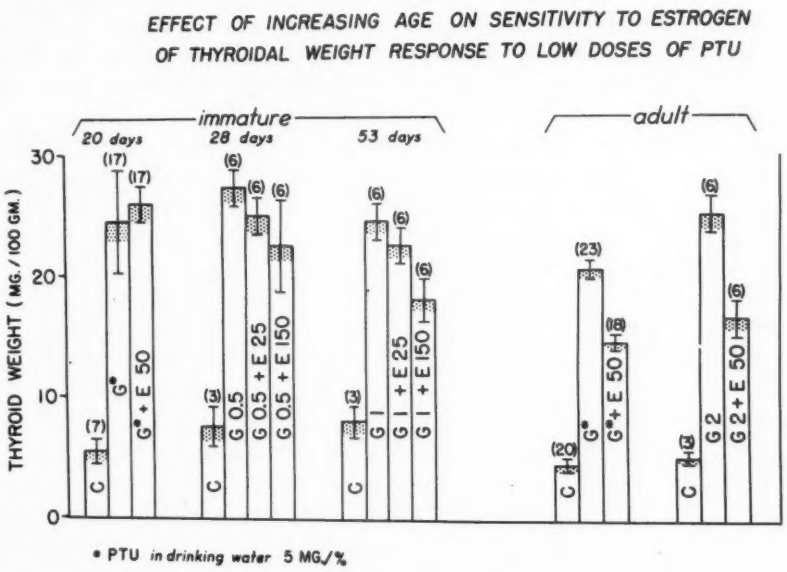


FIG. 1. Thyroid weights of adult and immature Vanderbilt rats given low doses of goitrogen, alone or in combination with estrogen, for 20 or 21 days. *Legend*: G = daily dose of propylthiouracil in mg.; E = daily dose of α-estradiol benzoate in micrograms; C = untreated, oil-injected, and estradiol-treated controls (pooled). Thyroid weights indicated by height of bars; dashes through the single vertical line at top of bar indicate standard error; figures in parentheses indicate number of rats in group. (In Fig. 2 and Fig. 3, double lines through the single vertical line intersecting top of bar indicate the range of the values, rather than the standard error. In some cases both data are given.)

injection, at a comparable dose level (2 mg./day), exhibited a somewhat greater degree (25.4 ± 1.35 mg./100 gm.) of thyroid enlargement. In both cases the concurrent administration of EB (50 μg./day) significantly ($p < .001$) lessened the degree of response to the goitrogen (by 29% and 34%, respectively). However, in all the PTU treated rats, both EB treated and control, the glands appeared frankly goitrous. Microscopically, they were extremely hyperplastic, with columnar epithelial cells surrounding,

and often folding in upon, acini which were devoid of colloid. The only evident distinction between the goiters of the two groups was one of size.

The same estrogen treatment (50 μg./day) failed completely to alter the goitrous response of 17 immature (20-day old) female Vanderbilt rats given PTU *per os* (Fig. 1), in spite of the fact that this dose of EB was about three times as great, on a body weight basis, as that which was effective in the adults. In slightly older (28 and 53 days at the onset) immature Vanderbilt rats, to which PTU in low dosage (0.5 or 1.0 mg./day) was given by injection, EB appeared to be only slightly effective, if at all, at a 25 μg./day level, and was less effective at 150 μg./day than was 50 μg./day in the adults. The inhibitory influence of EB upon goiter size was not statistically significant in either of these groups, although the data in Figure 1 suggest an incipient sensitivity of the older immature rats to this effect.

Effect of EB on thyroid response to higher dose levels of PTU. In an attempt to ascertain the mechanism by which EB exerted its effect upon the thyroid response to goitrogen administration, we investigated the influence of EB on male and female Sprague-Dawley rats given 1, 10 and 30 mg. of PTU/day for 21 days. The results are shown in Figure 2. The goitrogenic potency of the 10 and 30 mg. daily doses of PTU was not impaired at all by the 50 μg. dose of EB, although at the 1 mg. dose level this amount of the estrogen produced the same pronounced effect as in the previous studies. Even a higher dose of EB (150 μg./day) did not appear to alter goiter development appreciably in the male rats given 30 mg./day of PTU, although it may have been slightly effective in the females (Fig. 2 and Fig. 3).

Strain and sex variations. In Figure 3 are indicated the variations in goitrous thyroid weights of control and estrogen treated, PTU-injected, male and female rats of the three strains used. Although some of these data were collected at different times of the year from rats on different basic diets, this strain difference in order of response to PTU has been confirmed in subsequent studies[3] in which Wistar and Sprague-Dawley rats were experimented upon simultaneously. There appears to be a somewhat greater sensitivity among the female rats (Fig. 2 and Fig. 3) both to PTU and to EB. Because of the smaller size of the females, however, the administered dosages of these drugs probably reached higher tissue concentrations in rats of this sex. Regardless of any quantitative differences, the effects of PTU, alone and in combination with EB, were qualitatively alike in both sexes and all three strains.[3]

---

[3] In a subsequent study (unpublished) Wistar rats have been found to respond to the inhibitory influence of EB upon goiter development at low PTU dosage levels. However, they did not develop goiters as large as did Sprague-Dawley rats on an identical regimen.

DISCUSSION

Since it has recently been shown (3) that inanition during estrogen treatment results in an impairment of thyroid compensatory hypertrophy, the question of whether inanition might also account for the effect of estrogen in the present experiments needs to be considered. Estrogen treatment failed to alter the thyroidal enlargement induced by high doses of goitrogen, although the animals receiving it exhibited weight loss as great as those

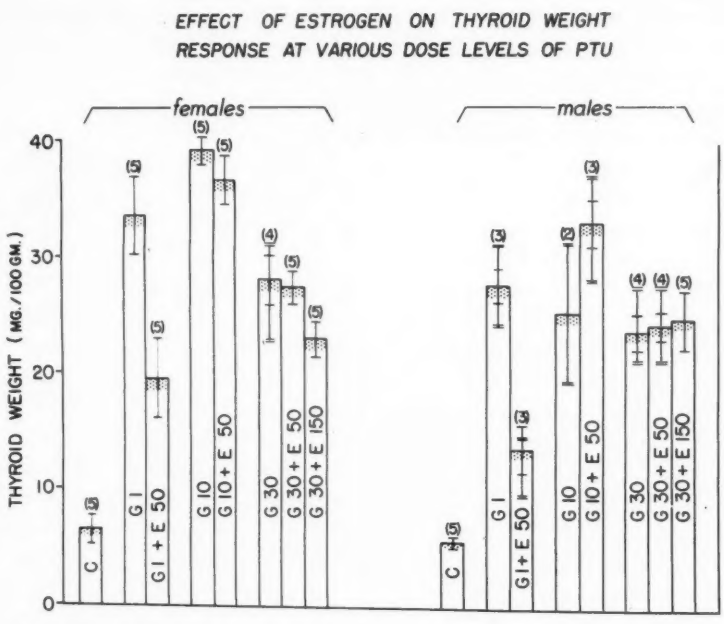


FIG. 2. Thyroid weights of adult male and female Sprague-Dawley rats given 1, 10 and 30 mg. of PTU/day (alone or together with estrogen) for 21 days. For interpretation of symbols, see legend for Fig. 1.

treated with low doses of goitrogen and the same amounts of estrogen in which the hormone did reduce goiter size. Therefore, inanition could not have played a major role in producing the phenomenon reported in this paper.

The thyroid weight data in these experiments agree with the findings of Chamorro (4) and Kopf (5) that large doses of various estrogens reduced, but did not abolish, the thyroidal enlargement in response to relatively small amounts of goitrogen. Histologically, the estrogen-inhibited goiters appeared maximally stimulated, as Kopf reported. This is in keeping with the observation of Rawson and Money (6) that, in response to thyrotropic stimulation, colloid discharge and cellular hypertrophy precede any gross

increase in thyroid weight. In view of this sequence of events, it is difficult to reconcile Chamorro's description of an unstimulated microscopic structure with the 50% increase in weight which he reported for the thyroid glands of rats treated with both estrogen and goitrogen.

The fact that the inhibitory effect of estrogen has been manifested only with low (1–2 mg./day) doses of PTU, or of methylthiouracil (5), may in part explain the failure of Desclin and Ermans (7) to encounter it. The

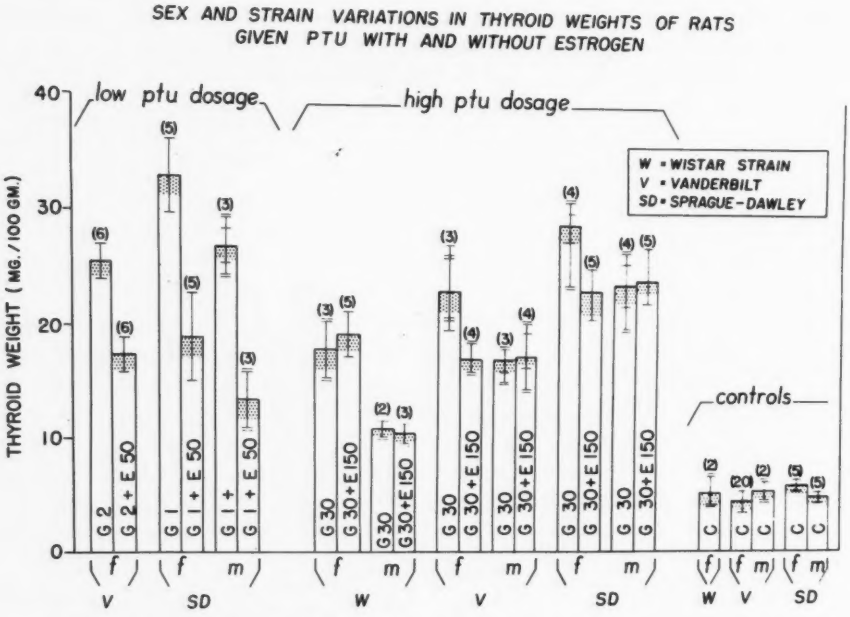


FIG. 3. Thyroid weights of adult male and female rats of three strains (see box for symbols used) given various doses of PTU and estrogen. Dosage indicated by symbols in Fig. 1.

failure of the hormone to depress the thyroidal response to 25 mg. of PTU/day (7) actually agrees with the present findings. However, Desclin also had similar results with thiouracil (8), a far less potent goitrogen. Although 25 mg./day of thiouracil might not have been sufficient to mask an inhibitory action of estrogen, as would this same quantity of PTU, another factor is noteworthy in Desclin's experiments. His control rats had thyroid weights about twice as large as those in our experience, suggesting the presence of an environmental, perhaps dietary (9), strumigenic factor. It is certainly possible that a summation of two weak thyroid stimulatory effects could have had the same masking effect as a single strong one.

The inhibitory effect of estrogen on goiter induction might be mediated by direct depression of either (a) pituitary thyrotrophin secretion or (b) the thyroidal hyperplastic response to this trophic hormone. In addition, a third possibility exists. There is experimental evidence that estrogen may elevate the iodine concentrating activity of the thyroid gland (10), and that this action of the hormone is a direct one (1, 2, 11). If this stimulation of iodine uptake were accompanied by an augmented production of thyroid hormone, the latter would act to inhibit the secretion of thyrotrophin. In a situation where the formation of thyroid hormone may be incompletely blocked, as by low doses of goitrogen, such heightening of the physiologic activity of the thyroid gland by estrogen could result indirectly in a diminution of the morphologic signs of stimulation. In keeping with this hypothesis is Kopf's finding that the depression of oxygen consumption by methylthiouracil treatment did not occur in the animals given EB concomitantly (5). The failure of EB to alter the thyroidal response to higher doses of PTU suggests that amounts of goitrogen which inhibit the biosynthesis of thyroid hormone more effectively may eliminate the opportunity for estrogen to act in this way. However, the presently available data do not permit any decision as to whether this interpretation of the estrogen inhibition of thyroid enlargement reported here is correct, and the many well-known effects of estrogens upon pituitary structure and function make the first possibility mentioned an equally likely one.

We are unable to explain the failure of estrogen to inhibit goiter development in weanling rats and its relative inefficacy in slightly older rats. Since this estrogen effect develops in adults of either sex, and occurs with doses which strongly inhibit pituitary gonadotrophin secretion, it does not seem probable that it is an effect which depends upon the concomitant secretion of endogenous estrogen. The stimulatory effect of estrogen upon thyroidal iodine uptake does not appear to depend upon the adrenals (2, 11) or gonads (11). It remains to be seen whether these glands are necessary for estrogen to inhibit goiter induction.

SUMMARY

α-estradiol benzoate (50μ g./day) was found to impair, but not abolish, goiter development in adult male and female rats given low doses of propylthiouracil for 20 or 21 days. This effect was not observed in immature (20-day old) rats. The estrogen did not alter thyroidal enlargement in adult rats given higher doses of the goitrogen. Possible mechanisms for this phenomenon have been discussed.

Acknowledgment

This study was initiated under the guidance of Prof. J. M. Wolfe. We wish to express our appreciation of his encouragement throughout the investigation.

REFERENCES

1. Noach, E. L.: *Acta endocrinol.* **19**: 127. 1955.
2. Noach, E. L.: *Acta endocrinol.* **19**: 139. 1955.
3. Clifton, K. H. and R. K. Meyer: *Endocrinology* **58**: 681. 1956.
4. Chamorro, A.: *Compt. rend. Soc. Biol.* **143**: 1540. 1949.
5. Kopf, R.: *Arch. exper. Path. u. Pharmakol.* **209**: 58. 1950.
6. Rawson, R. W. and W. L. Money: *Rec. Prog. Horm. Res.* **4**: 397. 1949.
7. Desclin, L. and A. M. Ermans: *Ann. endocrinol.* **12**: 238. 1951.
8. Desclin, L.: *Ann. endocrinol.* **13**: 433. 1952.
9. Halmi, N.S.: *Anat. Rec.* **112**: 17. 1952.
10. Soliman, F. A. and E. P. Reineke: *Am. J. Physiol.* **178**: 89. 1954.
11. Feldman, J. S.: *Am. J. Physiol.* **184**: 369. 1956.

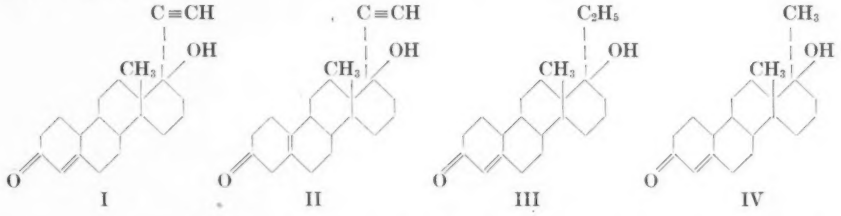
STUDIES OF THE BIOLOGICAL ACTIVITY OF CERTAIN 19-NOR STEROIDS IN FEMALE ANIMALS[1]

GREGORY PINCUS, M. C. CHANG, M. X. ZARROW[2]
E. S. E. HAFEZ[3] AND ANNE MERRILL

From the Laboratories of The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

INTRODUCTION

IN PREVIOUS publications a number of compounds have been demonstrated as inhibitors of ovulation in rabbits and rats (1, 2, 3). As our standard of reference we have used progesterone, which appears to be effective also in women (4,5). Of especial interest are four compounds: 17α-ethinyl-19-nortestosterone (I), 17α-ethinyl-5(10)estraen-17β-ol-3-one (II), 17α-ethyl-19-nortestosterone (III), and 17α-methyl-19-nortestosterone (IV).



These compounds have been suudied for: (a) progestational activity in rabbits, (b) deciduomagenic capacity in rats, (c) estrogenic activity, (d) ovulation-inhibiting activity in rabbits and rats, and (e) effects on development of the ovulated ovum.

Progestational activity in rabbits

The progestational activity of the various compounds was tested by the Clauberg (6) method. Immature, grey Chinchilla, female rabbits weighing approximately 1 kg. were used in this study. The rabbits were injected daily with 5 μg. of estradiol for 6 days. Following the estrogen priming, the rabbits were injected with the various progesterone-like compounds for 5 days along with 0.5 μg. of estradiol daily for 5 days. The rabbits were killed 24 hours after the last injection and the uteri removed and sectioned

Received August 20, 1956.

1 Investigations described in this paper were aided by grants from the Planned Parenthood Federation of America and G. D. Searle & Company.

2 Present address: Department of Zoology, Purdue University, Purdue, Illinois.

3 Present address: Faculty of Agriculture, Cairo University, Cairo, Egypt.

for histological examination. The progestational state of the uterus was then evaluated according to the McPhail (7) technique. All hormones were dissolved in oil or suspended in tween 80 and the injections were given intramuscularly in a volume of 0.1 ml.

In Table 1 we present the comparative assays of these compounds. The table demonstrates that full effectiveness of progesterone is attained at a total dosage of 2.0 mg. in either vehicle. Considering order of magnitude

TABLE 1. THE EFFECTIVENESS OF CERTAIN 19-NOR STEROIDS IN THE CLAUBERG TEST COMPARED WITH PROGESTERONE

Compound	Vehicle	Total dose, mg.	Response
—	Oil	0.5 ml.	—
—	Suspension	1.0 ml.	—
Estradiol	Oil	0.5 μg.	—
Progesterone	Oil	0.2	±
Progesterone	Oil	0.5	1+, 1+
Progesgerone	Oil	1.0	3+
Progesterone	Oil	2.0	4+
Progesterone	Suspension	0.2	1+
Progesterone	Suspension	1.0	2+
Progesterone	Suspension	2.0	4+
I	Suspension	0.02	—
I	Suspension	0.05	±
I	Suspension	0.1	3+
I	Suspension	0.2	4+
I	Suspension	0.5	4+
I	Suspension	1.0	4+
I	Suspension	5.0	4+
II	Suspension	0.5	±
II	Suspension	1.0	2+
II	Suspension	2.0	3+, 4+, 4+, 1+
II	Suspension	4.0	4+
II	Tablet (oral)	50.0	1+
II	Tablet (oral)	100.0	2+
III	Oil	0.01	±
III	Oil	0.02	±, ±
III	Oil	0.1	3+
III	Oil	0.2	4+
III	Oil	0.5	4+
III	Oil	1.0	4+
IV	Suspension	0.02	1+
IV	Suspension	0.1	3+, 2+

I, III and IV are approximately ten times as active as progesterone and II, albeit with some irregularity, is approximately as active as progesterone the activity of II by mouth is considerably reduced, by a factor of 50 to 100 times.

In view of the foregoing, it was our expectation that these compounds would be efficient in maintaining implantation in ovariectomized rabbits carrying fertilized eggs. The demonstration by Allen (8) and Pincus and Werthessen (9) that progesterone in dosages from 1.0 to 1.5 mg. per

day is sufficient to establish implantation in the rabbit ovariectomized one day post coitum led us to repeat their experiments using post-partum females mated twice to bucks of known fertility. To ensure ovulation 12 r.u. of a sheep pituitary extract was injected intravenously into the does at the time of mating. Twenty-four hours later the females, in groups of four, were ovariectomized and the number of corpora lutea were counted. Daily injections of the steroids in oil were made, and at ten days post coitum the animals were sacrificed and the uteri were examined to determine the number of implantation sites and the condition of the fetuses.

The frequency and number of implantations obtained with two of the three 19-nor steroids was surprisingly low. In Table 2 we present the data of the series exhibiting successful implantation. The progesterone was

TABLE 2. IMPLANTATION IN OVARIECTOMIZED RABBITS RECEIVING PROGESTINS

Compound	Dosage, mg./day	No. of animals	Av. No. of ova ovulated	Av. No. of implantations	% implanted	% degenerating
Progesterone	from 0.5 to 1.5	4	13.0	5.5	42	23
Progesterone	from 0.3 to 5.0	4	11.3	4.0	36	14
Progesterone	from 0.2 to 10.0	3	9.3	3.3	36	11
Progesterone + estradiol (E)	from 0.5 to 1.5 + 1/1000 E	4	12.0	4.0	33	13
Progesterone + estradiol (E)	from 0.3 to 5.0 + 1/1000 E	4	12.5	3.5	28	8
Progesterone + II	5.0+0.04	4	12.0	3.7	31	0
III	2.0	4	10.3	5.2	53	10
III	10.0	4	10.3	5.5	51	0
III + estradiol	2.0+0.002	3	10.0	2.0	20	10
TABLE 2A						
I #2	0.25	2	13.0	11.0	85	55
I #2	2.0	4	7.0	0.5	8	0
I #2	10.0	4	9.8	1.5	15	0

injected in increasing dosage from day to day since we had previously demonstrated (10) that a constant daily dosage of 2 mg. per day regularly induced implantation of approximately 20% of the ova ovulated. In this same test I was administered in daily dosages of 0.2 and 1.0 mg. per day, and in the following combinations 0.2 mg.+0.2 and 0.4 μg. of estradiol, 1.0 mg.+1 and 2 μg. of estradiol, 0.2 mg.+0.25, 1.0 mg. and 4.0 mg. allopregnanedione and 1.0 mg.+0.25, 1.0 mg. and 4.0 mg. allopregnanedione; in no instance was implantation observed. Similarly II in daily dosages of 0.2 and 1.0 mg., of 2.0 mg.+4 μg. estradiol, of 0.2 mg.+5 mg. of progesterone and 2.0+1 mg. of progesterone was ineffective. IV has not been studied in this test. It would appear from the data of Table 2

that III is potent as a maintainer of implantation, and that its efficiency in this respect may be reduced by estrogen. The efficiency of I and II is not established by our data, but it appears certain that it does not parallel their efficiency in the Clauberg test; in fact the data suggest that II may act as an antagonist of progesterone in the implantation process. We have previously shown that progesterone given in single daily doses of 2 or 25 mg. does not succeed in effecting implantation of more than 23% of the ova on the average. Some increase in this percentage (up to 42%) appears to be achieved by staggering the daily dose, and III in constant daily dose (2 or 10 mg.) seems to be more successful in maintaining a high implantation rate than progesterone. Nonetheless the implantation rate of 93% observed in normal pregnancy which we have previously observed (10) is not attained by any replacement regime thus far attempted.

As is demonstrated below, certain preparations of I seemed to have a certain amount of estrogen as a contaminant. Accordingly we obtained a preparation especially prepared to be estrogen-free (I #2 of Table 4). This preparation was injected in the dosages indicated in Table 2A. These data suggest implantation-stimulating activity at low dose but relative ineffectiveness at high dose. Among the ova implanted at the low dose, however, there was a significant (55%) degree of degeneration suggesting marginal ability of I to sustain implantation.

Deciduoma in the rat

Decidual activity was tested in the rat using the classical techniques except that two types of injection schedules were employed. Pseudopregnancy was induced by electrical stimulation of the uterine cervix of the rat in estrus. Each animal was castrated 5 days later and the right uterine horn traumatized. The animals were then given a single injection and killed 3 days after the trauma or given 3 daily injections and similarly killed 3 days after the trauma. Ten animals were used for each dosage of each compound tested. The various steroids were dissolved in oil or suspended in Tween 80 and a 0.1 ml. volume used at each injection. Both uterine horns were removed and weighed separately. The degree of stimulation was expressed as the per cent increase in the weight of the traumatized horn over the control horn.

The data presented in Table 3 demonstrate (a) the ineffectiveness of I and II, at least in dosages comparable to those found effective with progesterone, (b) the effectiveness of III and IV at least of the same order of magnitude as progesterone. The results of this test seem to parallel to some extent those obtained in the study of implantation efficiency (Table 2).

Estrogenic activity

Thus far we have studied the estrogenic activity of three of these compounds: I, II and IV have been tested in the Rubin assay (11) using the

TABLE 3. THE EFFECTIVENESS OF CERTAIN 19-NOR STEROIDS IN THE RAT DECIDUAL TEST

Compound	Vehicle	Type of dose	Total dosage, mg.	% increase in weight of traumatized horn
Progesterone	Oil	Single	5	150 ± 15
Progesterone	Oil	Single	10	276 ± 25
Progesterone	Suspension	Single	10	193 ± 33
Progesterone	Oil	Multiple	2	261 ± 38
Progesterone	Oil	Multiple	3	344 ± 21
Progesterone	Oil	Multiple	5	148 ± 35
Progesterone	Suspension	Multiple	0.5	44 ± 13
Progesterone	Suspension	Multiple	2	111 ± 42
I	Suspension	Single	10	30 ± 12
I	Suspension	Multiple	0.3	28 ± 2
II	Suspension	Single	10	8 ± 2
II	Suspension	Single	10	7 ± 6
II	Suspension	Multiple	15	7 ± 2
III	Oil	Single	10	272 ± 44
III	Oil	Multiple	0.05	8 ± 3
III	Oil	Multiple	0.3	43 ± 12
III	Oil	Multiple	0.5	64 ± 12
IV	Suspension	Multiple	0.5	18 ± 1.3
IV	Suspension	Multiple	3.0	190 ± 31

uterine weight of the immature (21 to 22 days old) mouse. In Table 4 we present the data obtained on two samples of I, or which #2 is designated as "highly purified," and on similarly "highly purified" sample of II and IV. It is clear that the two samples of I differ in estrogen activity, #1 being approximately 8 to 10 times as active as #2. Both are, however, considerably less active than the estrone standard. Quantitative comparison with the standard is not possible, however, since I has, in each experiment, a slope constant in the dosage response curve significantly different from that of estrone (Fig. 1). A rough estimate leads to an activity 1/800th to 1/1000th of estrone for #1 and 1/6400th to 1/10,000th for #2. These data suggest an estrogen contaminant in each sample which has been markedly reduced in #2; alternatively, #1 has such a contaminant whereas #2 exhibits an intrinsic uterus-stimulating activity of I with no contaminant.

In Figure 1 we plot the dosage; response data for the various assays of Table 4, along with assay values for progesterone. The most purified sample of I and IV have very similar dosage: response curves whereas II has a higher slope and a lower threshold. II, therefore, appears to have a different order of estrogenic activity; this may be conferred by the shift of the double bond to the 5:10 position. Again the slope of its curve differs from that of the standard but a rough estimate of 1/40th to 1/80th of estrone's activity may be made.

In the vaginal cornification test 120 to 200 gm. female rats were castrated and one week later half of them were injected with estradiol-17β and the other half with II; one week later the two groups were interchanged. Vaginal smears taken morning and night for $2\frac{1}{2}$ days beginning forty-one hours after the first of three injections of sesame oil were stained with Giemsa, and marked positive if only nucleated and epithelial cells

TABLE 4. THE UTERINE RESPONSE (UTERINE WEIGHT IN MG.)/(BODY WEIGHT IN GM.) OF IMMATURE FEMALE MICE TO CERTAIN 19-NOR STEROIDS

Expt.	Compound	Total dose, μg.	No. of mice	Mean uterine ratio ±S.E.*
A	Control	—	11	0.88±0.065
	Estrone	0.025	14	1.22±0.050
	Estrone	0.05	14	1.80±0.071
	Estrone	0.1	14	3.37±0.231
	Estrone	0.2	13	4.92±0.344
	I #1	20	13	2.26±0.065
	I #1	40	13	2.50±0.079
	I #1	80	13	2.89±0.111
	I #1	160	13	3.43±0.115
B	Control	—	12	1.24±0.006
	Estrone	0.025	12	1.77±0.125
	Estrone	0.05	12	2.39±0.125
	Estrone	0.1	12	2.94±0.276
	Estrone	0.2	11	4.83±0.276
	I #2	15	10	1.61±0.054
	I #2	45	11	1.74±0.071
	I #2	90	12	1.86±0.075
	I #2	270	9	2.17±0.111
C	Control	—	11	1.05±0.089
	Estrone	0.025	11	1.15±0.051
	Estrone	0.05	11	1.73±0.083
	Estrone	0.1	9	3.20±0.380
	Estrone	0.2	11	4.87±0.255
	II	2	11	2.90±1.71
		20	10	4.57±0.234
D	Control	—	15	1.07±0.064
	Estrone	0.025	10	1.38±0.085
	Estrone	0.05	9	1.68±0.105
	Estrone	0.1	10	2.67±0.162
	Estrone	0.2	10	4.20±0.349
	IV	2	10	1.31±0.081
	IV	20	8	1.51±0.067
	IV	100	10	2.05±0.092
	IV	1000	10	2.61±0.104
	Progesterone	100	10	1.75±0.079
	Progesterone	500	10	1.47±0.104
	Progesterone	1000	10	1.87±0.075

* S.E. = standard error.

were present. Scanty smears or smears with 5% or more leucocytes were considered negative. The data of Table V demonstrate some estrogenic activity of II. On the basis of the dose-response curve the 50% response is at 0.065 μg. for estradiol and at 13.5 μg. for II, indicating 1,200th of the activity of estradiol for II, but it should be noted that II has a curve which is less steep than that of estradiol. Since estrone in this assay is approximately 1/5th as active as estradiol, the order of magnitude of activity is that observed in the Rubin test.

Drill and Saunders (12) report no detectable estrogenic activity of III at the 10 μg. level in the Rubin test, but have since found it to have less than 1/200th the activity of estrone. They find it inactive in the vaginal cornification test (13).

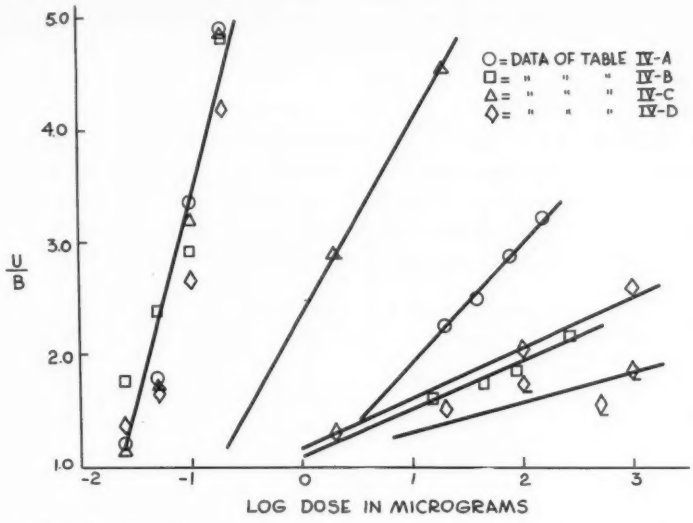


FIG. 1. Relationship between the logarithm of the dose and the uterine weight; body weight ratio in immature female mice receiving estrogens, progesterone and various 19-nor steroids.

Ovulation inhibition in rabbits

Ovulation inhibition in rabbits has been studied using post-partum females which are injected with the test substance twenty-four hours before mating to a fertile male (1). Laparotomy is performed twenty-four hours after mating to determine the presence or absence of ovulation points on the ovary. In Table 6 we present the data on frequency of ovulation in a control series of 62 post-partum females and in females receiving various dosages of the 19-nor steroids. It is clear that they are all potent ovulation inhibitors, and they all have roughly the same order of magnitude of activity. Thus one to two mg. of each compound is practically 100% effective as an ovulation inhibitor, but compound I and II appear to be active at a somewhat lower level. We have previously reported that 1–2 mg. is

TABLE 5. THE ESTROGENIC ACTIVITY OF 17-ETHINYL-ESTRAENEOLONE (II) IN THE VAGINAL CORNIFICATION TEST

Substance	Total dose, μgs.	No. of rats used	Vaginal cornification	
			No. positive	% positive
Estradiol	0.1	10	10	100
Estradiol	0.075	20	19	90
Estradiol	0.05	10	1	10
II	20.0	10	8	80
II	15.0	20	7	65
II	10.0	10	2	20

the lowest level at which progesterone is active (3, 4). Oral administration of these compounds also leads to effective ovulation-inhibition.

Ovulation inhibition in rats

The methods which we have used for the study of the inhibition of ovulation and early development in rats have been previously described (2, 4). Thus far I, II and III have been administered to mature breeding female rats which are placed with fertile males at the time of initial (or only) administration. We have found that the significant indicator of anti-

TABLE 6. THE EFFECTS OF CERTAIN 19-NOR STEROIDS ON OVULATION IN THE POST-PARTUM RABBIT. (ALL BY SUBCUTANEOUS INJECTION UNLESS OTHERWISE NOTED)

Compound	Dosage	No. of animals	% ovulating
Vehicle	—	62	82 ± 4.88
I	0.1	4	50
	0.25	4	0
	0.5	8	0
	2.0	4	0
	10.0	4	0
	5 (oral)	4	0
II	0.1	5	40
	0.2	5	40
	1.0	5	0
	5.0 (oral)	5	20
	10.0 (oral)	5	40
III	0.2	4	25
	1.0	5	20
	5.0	5	0
	10.0 (oral)	5	40
IV	0.1	4	50
	0.5	4	25
	2.0	4	0
	5.0 (oral)	3	33

fertility effect is the latent period between cohabitation with the male and the successful mating that culminates in pregnancy. This is evident in the data of Table 7 which demonstrate significant effects of I, II and III upon this measurement. I and II are clearly active both by injection and by oral administration and II appears to be somewhat more active than I, particularly by the oral route. III is active by the oral route at the 5 mg. per day dose, but appears to be somewhat less effective than I or II. A significant increase in number of corpora lutea followed the subcutaneous injection of 1 mg. of II, but this failed to occur at higher doses or under any other regime. Similarly, a significant increase in the number of normal embryos occurred following two weeks of feeding of I, but whether this signifies some action to conserve fetuses is difficult to deduce, particularly since this does not occur under other regimes of administration. What the data do suggest generally is that the pregnancy following on the period

TABLE 7. THE EFFECTS OF 19-NOR STEROIDS ON MATING AND PREGNANCY IN THE RAT

Compound	Dosage regime	Dose, mg.	No. of rats	Days between cohabitation and successful mating	Average No. of corpora lutea	Average No. of normal embryos	% of ova failing to implant	Fetal degeneration (as % of total embryos)
Control	—	—	59	4.7±0.57	12.2±0.41	9.4±0.37	9.0	6.9
I	S.I.*	10	11	*38.3±3.12*	13.6±1.19	8.6±0.94	10.0	29.7
	S.F.†	10	12	*9.3±1.48*	13.4±0.87	11.6±1.22	11.7	2.8
	S.F.	25	11	*16.3±1.96*	12.7±0.73	11.1±0.96	10.1	2.4
	F2×2‡	5	11	*17.5±1.50*	14.4±1.11	12.1[1]±0.68	13.9	2.2
II	S.I.	1	12	*13.7±1.43*	15.6[1]±1.21	11.8±0.97	15.5	10.8
	S.I.	2	12	*21.8±2.04*	14.0±0.78	11.3±0.51	8.3	12.3
	S.I.	5	12	*17.8±1.67*	11.7±0.66	10.3±0.65	3.6	8.9
	S.F.	2	10	10.4±3.39	14.7[1]±0.80	12.2±1.30	15.6	1.6
	S.F.	5	12	*16.5±2.66*	13.1±0.77	9.6±0.95	12.1	16.7
	F2×2	2	12	*16.3±1.16*	12.7±0.54	10.3±1.17	8.6	11.5
III	F2×2	1	10	9.2±2.61	12.8±0.49	11.6±0.65	6.5	3.5
		5	10	*9.5±1.96*	11.6±1.09	10.3±1.09	11.0	2.6

[1] Significantly higher than control value.
* S.I. = single subcutaneous injection.
† S.F. = single feeding.
‡ F2×2 = feeding twice a week for two weeks.
Italicized values differ significantly from the control.

of delay proceeds normally; a single possible exception is the high percentage (29.7) of fetal degeneration following the 10 mgm. injection of I, but this may be adventitious since it is not observed under the other regimes.

To test the effects of prolonged administration II was given in 2 mg. dose by stomach tube every other day over a period of 70 days to mature adult nonpregnant female rats. These animals were divided into two groups, one of which was caged with fertile males at 18 days, and the other at 39 days following the initiation of medication. Control females were similarly caged with males at these two intervals following the initiation of administration of the oil vehicle. Fertility records were kept and the data of these records are presented in Table 8. In the first control group the mean latent period to conception was 28 days. This is quite unusual (*cf.* Table 7 controls) and immaturity of the male is suspected since vaginal sperm was not observed in these animals until $3\frac{1}{2}$ to $4\frac{1}{2}$ weeks after caging with the male. Nonetheless, on comparison with the experimental animals which exhibited an average delay of 78 days to conception a

TABLE 8. THE EFFECTS OF THE ORAL ADMINISTRATION OF 17-ETHINYLESTRAENEOLONE UPON THE REPRODUCTIVE BEHAVIOR OF ADULT FEMALE RATS. EACH EXPERIMENTAL ANIMAL RECEIVED 2 MG.-IN-OIL BY STOMACH TUBE EVERY OTHER DAY, EACH CONTROL ANIMAL RECEIVED AN EQUAL AMOUNT OF THE OIL SOLVENT EVERY OTHER DAY

Type of animal	No.	Total dosage, mg.	Caged with males after	Latent period to conception, days	Sterile period following medication, days
Control	4	—	18 days	28±1.63	—
Experimental	4	70	18 days	78±0.91	26
Control	5	—	39 days	7.4±0.71	—
Experimental	4	70	39 days	59.5±4.85	28.5

clearly significant difference is observed. In the second group the prompt conception of the control contrasts clearly with the 59.5 day delay to conception in the medicated animals. In Table 8 we have calculated the mean period of sterility ensuing following discontinuance of medication in the two experimental groups, 26 and 28.5 days respectively. This is calculated as follows: in the first group the animals were with males for 52 days during medication; they conceived after 78 days of cohabitation with the male—therefore $78-52=26$ days of sterility followed discontinuation of regular medication. The remarkable similarity in length of the post-medication sterile period with the two groups suggests an inhibitory effect from which recovery is had regardless of the length of exposure to males, i.e. it is intrinsic to the rate of readjustment of the reproductive process of the medicated animals. It should be noted that mating took place from time to time with the medicated animals as judged by the presence of sperm in the vaginal smears which were taken daily. In spite of such matings sterility supervened.

Effects on ovum development

We had assumed that the mechanism of this effect in the rat is due to inhibition of ovulation. It may, however, be ascribable to failure of fertilization or of implantation of the fertilized ovum. To examine this point six female rats were given a single injection of 10 mg. of II and caged with fertile males 24 hours later. One of them mated within 24 hours (as judged by the presence of sperm in the vagina) and four days later she was sacrificed. Fifteen corpora lutea were found in the ovaries and 15 eggs in the oviducts, none of them fertilized. A second female mated twice, once within 24 hours and again within 72 hours of caging; four days after the second mating we found 20 corpora lutea and 20 unfertilized eggs in the oviducts. A third female mated on three successive occasions, at 3, 6 and 9 days following caging; four days after the third mating we found 14 corpora lutea and 14 unfertilized ova. A fourth female mated fifteen days after caging, and five days later we found 18 corpora lutea and 18 unfertilized ova. The remaining two females failed to mate over a 30-day period and on sacrifice gave no evidence of recent ovulations. These data suggest, therefore, a dual effect, at least of II: (a) an inhibition of fertilization in animals mating shortly after its administration and (b) an inhibition of ovulation for a greater or lesser period of time; where the period of time is lesser, e.g., 15 days, the fertilization-inhibiting effect may still persist.

In the case of the rabbit the ovulation-inhibiting effect is plain. Nonetheless, a possibility that effects on the ovum may obtain still exists. Accordingly, we undertook the following experiment. Ten post-partum rabbits were injected with 5 mg. of II per day for three successive days, and on the fourth day they were mated to proven fertile males. Immedi-

ately after mating they received an intravenous injection of 12 rat units of a sheep pituitary gonadotrophin to ensure ovulation. All ten did in fact ovulate as determined by laparotomy 24 hours later. One fallopian tube was removed from each of three females at this laparotomy, the ova were flushed from the tubes and examined for evidence of fertilization. All of the ova from two females were clearly fertilized, and some of the ova from the third were fertilized. It would therefore appear that in the rabbit II does not inhibit fertilization as it does in the rat. The uterus on the remaining side of two of the rabbits was examined for blastocysts 5 days later,

TABLE 9. A COMPARISON OF ESTIMATED MINIMAL ACTIVE DOSES OF PROGESTERONE AND THE 19-NOR STEROIDS IN SIX TESTS OF ACTIVITY

	M.E.D. in Clanberg assays, mg.	Minimum utero-trophic dose in mice, μg.	Minimum deciduo-magenic dose in rats mg.	Minimum oral anti-fertility dose in fertile rats, mg.	Minimum ovulation-inhibiting dose, mg.	Minimum implan-tation sustaining dose in rabbits, mg./day
Progesterone	1–2	87	2	5–10+	1–2	0.5–1.5
I	0.1–0.2	29	>10	5	0.25	0.25*
II	2–4	0.24	>15	2	>0.2<1.0	>2.0
III	0.1–0.2	±100†	0.5	5	>1.0<5.0	2.0
IV	0.1+	19	2	not studied	0.5–2.0	not studied

* Less active at higher dose.
† Data of Drill, Saunders and Edgren (11).

but none were found. Furthermore, the remaining rabbits were allowed to go to term, but no young were born, and palpitation of the uteri at intervals gave no evidence of living embryos. In the rabbit, then, this compound may act to inhibit normal development of the fertilized egg. We are examining this point in further studies.

DISCUSSION

One of these 19-nor steroids, I, has previously been reported as an active progestin by conventional assay in rabbits (14) and in women (15, 16). On closer scrutiny of the array of tests which we have employed complete characterization of these compounds as progestins becomes rather difficult. This is illustrated in Table 9, which compares the calculated minimum effective doses of the various 19-nor steroids with those of progesterone in six tests of activity. The data on the mimimum uterotrophic dose for I, II and IV are derived from the extrapolation of the curves of figure 1 to a uterine weight:body weight ratio of 1.30. The remaining values are calculated from the data of this and previous publications. On a quantitative basis there is no complete parallelism. Thus, compound I which is approximately 10 times as active as progesterone in the Clauberg assay is clearly much less active as a deciduomagenic agent. Compound II

which is somewhat less active than progesterone in the Clauberg assay is many times more potent as a uterotrophic agent in the mouse, and is not detectably deciduomagenic. Compounds III and IV are the only ones which exhibit a rough parallelism to progesterone, but both are more active in the Clauberg and uterotrophic assays and approximately as active as progesterone in the other tests.

Compound II would appear to be uniquely distinguishable from the others on the basis of its high uterotrophic activity and also the fact that it is active in the rat vaginal smear assay. This gives to it a clear estrogenic activity in the conventional sense. It is a not very potent progestin compared to the other compounds, is either not at all or only feebly deciduomagenic and appears to be unable to sustain implantation in the dosages thus far employed. If we add to these findings the evidence for its inhibitory effects on the development of early ova, i.e., inhibition of fertilization in the the rat and of blastocyst implantation in the rabbit it might almost be denominated an estrogen. In the human female it does act upon the endomentrium as a progestin (5); it and II and III also appear to act as ovulation inhibitors (4, 5). Compound III, on the basis of our tests, would appear to be the most effective as a progesterone substitute. It has all of the qualitative activities of progesterone and especially in its dosage: response relationship in the uterotrophic assay it resembles progesterone quite markedly. Nonetheless in the human female it appears to be unable alone to sustain normal endometrial development (5).

Each of these compounds would appear to offer the possibility of acting as anti-fertility agents. Of them, Compound II would appear to be most promising for this purpose since it not only is an effective ovulation inhibitor but also may act to prevent development of the free ovum. The ratio of ovulation-inhibiting to ovum-inhibiting dosages has, however, not been established. Furthermore, the etiology of the period of sterility which we have observed following prolonged administration to the female rat requires elucidation.

SUMMARY

Four 19-nor steroids—17α-ethinyl-19-nortestosterone (I), 17α-ethinyl-5(10)estraen-17β-ol-3-one (II), 17α-ethyl-19-nortestosterone (III), and 17α-methyl-19-nortestosterone (IV) have been assayed in a series of tests designed to demonstrate progestational and/or estrogenic activity. All of them are active in the Clauberg test with I, III and IV having approximately ten times the activity of progesterone and II having about $\frac{1}{2}$ the activity of progesterone. All of them are active as ovulation inhibitors in the rabbit, I and II appearing to be somewhat more active than progesterone, III and IV of the same order of magnitude as progesterone. In the uterotrophic assay in the mouse I, III and IV have a dosage:response curve resembling that of progesterone whereas II has one more nearly

resembling that of estrone; II also has a much higher order of activity than the other compounds. The deciduomagenic activity of III and IV resembles that of progesterone, but neither I nor II has proven active in doses 5 to 7 times that of progesterone. Compounds I, II and III are effective anti-fertility agents in the female rat; I and especially II appear to be more active than progesterone when administered by injection; III has been tested only orally and appears to be about as active as I and somewhat less active than II. Compounds I, II and III have also been studied as maintainers of implantation in the rabbit; III has the order of magnitude of activity of progesterone, II has been inactive at a dosage at which progesterone is regularly active and I appears to be relatively ineffective over a wide dosage range although at low dosage (0.25 mg. per day) implantation of a larger percentage of ova occurs but with much accompanying fetal degeneration. No compound tested, including progesterone itself, regularly effects a normal percentage of implantation of fertilized eggs. Special studies with II indicate that (a) it acts as an inhibitor of fertilization in the rat, but not in the rabbit, (b) it prevents implantation or the maintenance of implants in the normally pregnant rabbit, (c) during a 70-day regime of injection of 2 mg. every other day in female rats conception does not occur although copulation may occur, and that the period of administration is followed by a 26 to 28-day period of sterility regardless of when the male is caged with the females.

Acknowledgments

We gratefully acknowledge the technical assistance of Mrs. A. Dorfman, Miss P. Longo, and Mr. P. Bodurtha.

REFERENCES

1. Pincus, G. and M. C. Chang: *Acta Physiol. Latino-Americana* **3**: 177. 1953.
2. Slechta, R. I., M. C. Chang and G. Pincus: *Fertility and Sterility* **5**: 282. 1954.
3. Pincus, G.: Aspects du Métabolisme des Steroides Hormonaux, Mason et Cie, Paris, 1955.
4. Pincus, G.: Proc. Vth International Planned Parenthood Conf. (in press).
5. Rock, J., G. Pincus and C. R. Garcia: Recent Progress in Hormone Research 13 (in press).
6. Clauberg, C.: *Zentr. Gynäkol.* **54**: 2757. 1930.
7. McPhail, M. K.: *J. Physiol.* **83**: 145. 1934.
8. Allen, W. M.: *Cold Spring Harbor Symposium on Quan. Biol.* **5**: 66. 1937.
9. Pincus, G. and N. T. Werthessen: *Am. J. Physiol.* **124**: 484. 1938.
10. Hafez, E. S. E. and G. Pincus: *Proc. Soc. Exp. Biol. Med.* **91**: 531. 1956.
11. Rubin, B. L., A. S. Dorfman, L. Black and R. I. Dorfman: *Endocrinology* **49**: 429. 1951.
12. Drill, V. A. and F. J. Saunders: In Engle and Pincus: Hormones and the Aging Process. Academic Press, New York. 1956.
13. Drill, V. A., J. F. Saunders and R. A. Edgren: Personal communication, 1956.
14. Hertz, R., W. Tullner and E. Raffelt: *Endocrinology* **54**: 228. 1954.
15. Tyler, E.: *J. Clin. Endocrinol. and Metab.* **15**: 881. 1955.
16. Greenblatt, R. B.: *J. Clin. Endocrinol. and Metab.* **16**: 869. 1956.

NOTES AND COMMENTS

THE EFFECT OF SYNTHALIN A ON BLOOD SUGAR AND PANCREATIC ALPHA ISLET CELLS OF THE FOWL*

The analysis of the cytotoxic effect of Synthalin A on the pancreas of experimental animals recently has strengthened the evidence in support of the secretion of a second pancreatic islet hormone, glucagon. Davis (1) was the first to demonstrate the selective cytotoxic action of Synthalin A on the alpha cells of the Islets of Langerhans of rabbits. Since then similar hydropic degeneration of the alpha cells has been reported in the Guinea pig (2), and in the rat (3).

Davis (1) also observed an initial rise in blood sugar of rabbits following a single subcutaneous injection of Synthalin A. The initial, transient, hyperglycemia has also been reported in greater detail by Fodden (4). Following the initial hyperglycemic phase, a subsequent hypoglycemia ensues, which, when the dosage is high enough, leads to fatal convulsions. This hypoglycemia has been reported by Davis (1) in rabbits and by von Holt *et al.* (5) in rats.

This paper reports some effects of Synthalin A in birds, and compares the fowl with the mammal in regard to these effects.

MATERIALS AND METHODS

Single comb White Leghorn roosters were used. The birds were obtained as day old chicks and used for the blood sugar study when 147–163 days-of-age. When treated, they ranged in body weight from 1.6–2.2 kg. The birds used for the histological study were 200 days-of-age when treated and weighed 1.6–2.3 kg.

The Synthalin A (decamethylenediguanidine) was administered in dosages of 1.0, 5.0, and 10.0 mg. per kg. body weight as fresh 1 per cent aqueous solution. All treatments consisted of a single subcutaneous injection in the neck region. The control birds were injected with 1.0 ml. of distilled water. Prior to time of treatment the birds were not fasted. Blood samples were obtained by the method of Opdyke (6) immediately before treatment and at intervals following the injection as listed in Table 1. The blood sugar was determined by the micromethod of Folin and Malmros as adapted for the photoelectric colorimeter by Horvath and Knehr (7).

One series of 10 birds was treated with 5 mg. Synthalin A per kg. body weight. These birds were killed by decapitation when convulsions began, and the pancreas, along with those from untreated controls, quickly removed and transferred to Bouins fixative. Sections of these glands were made at three microns of thickness and stained by the chrome haematoxylin and phloxine method of Gomori (8).

RESULTS

Blood Sugar Studies

The results of the effect of Synthalin A on blood sugar are given in Table 1 and a summary of the data is presented in Figure 1. The blood sugar value range for the birds before treatment was in agreement with that of previous investigators as reviewed by Sturkie (9).

The most striking results were obtained with the 10 mg./kg. Synthalin A dosage level. A hyperglycemia was noted 20 and 40 minutes after treatment that was significant at the 5% level of confidence as determined by the "t" test (10). Thereafter, an increasingly profound hypoglycemia ensued in all the birds and death occurred between 3.2 and 4.5 hours after injection. The death of the animal was always preceded 10 to 30 minutes by convulsions.

Received June 5, 1956.

* Contribution #615 from the Zoology Department, Indiana University.

TABLE 1. BLOOD SUGAR CHANGES IN THE FOWL FOLLOWING SUBCUTANEOUS INJECTIONS OF SYNTHALIN A. VALUES REPRESENT PERCENT CHANGE FROM PRE-TREATMENT LEVEL

Bird	Synthalin-A	Initial blood sugar[2]	Time after injection								Died at:
			20 min.	40 min.	60 min.	80 min.	100 min.	2 hrs.	2½ hrs.	3 hrs.	9 hrs.	
508	10 mg./kg.	185	+20.0	+20.0	+ 2.9	−10.9	−31.4	−44.0	−51.5			3.8 hrs.
504		170	+13.1	+ 4.4	− 6.9	−22.5	−40.0	−54.4	−58.8			3.2 hrs.
505		181	+19.3	+25.2	+25.2	+11.7	+ 5.3	−10.5	−25.2			4.5 hrs.
512		173	+ 3.9	+ 9.2	+ 1.3	− 1.3	−12.4	−18.3	−28.8	−50.0		4.2 hrs.
511		175	+22.0	+ 7.8	−14.8	−29.6	−42.6	−54.8	−67.8			3.2 hrs.
510		176	+16.7	+21.2	+ 4.5	+ 1.3	−14.1	−28.8	−50.6	−61.0		4.2 hrs.
	Mean (±S.E.)[1]		+15.8* (±2.7)	+14.6* (±3.5)	+ 2.0 (±5.5)	− 8.6 (±6.3)	−22.5* (±7.6)	−35.1** (±7.7)	−47.1** (±6.8)			
507	5 mg./kg.	175	+ 6.7	+ 9.7	− 0.6	− 5.5	− 9.1	− 6.7	−16.4	−20.6	—	8.5 hrs.
506		187	+11.3	+16.4	+10.4	+ 7.4	+10.4	+ 6.2	+ 2.3	+ 2.3	−23.8	16–20 hrs.
509		176	+ 9.6	+10.9	+11.5	+ 5.8	+ 5.8		+ 2.6	+ 9.8	−59.0	11.3 hrs.
352		175	+ 0.6	+ 2.3	− 4.6	−10.3	−17.2	−24.6	−28.0	−36.6	—	8.5 hrs.
351		189	+ 5.3	+ 4.2	+ 2.6	+ 1.6	− 2.1	− 1.6	− 2.6	+ 1.6	−31.2	12–24 hrs.
353		185	+13.0	+12.4	+ 3.2	+ 3.8	− 1.6	− 3.2	−11.9	−15.1	−59.0	12–24 hrs.
	Mean (±S.E.)		+ 7.8 (±1.8)	+ 9.3 (±2.2)	+ 3.7 (±2.5)	+ 0.5 (±2.7)	− 2.3 (±4.0)	− 6.0 (±5.1)	− 9.0 (±4.9)	− 9.8 (±7.1)		
349	1 mg./kg.	179	+ 3.3	+ 6.7	+ 1.7	+ 3.9		+ 3.3	+ 2.8	+ 4.5		
347		169	+12.4	+23.0	+16.0	+ 1.8		+ 8.9	+12.4	+ 5.3		
348		181	+ 4.1	0.0	+ 8.2	+ 1.8		+ 2.3	+ 3.5	+ 1.8		
502		178	+ 9.0	+10.7	+ 3.9	0.0		− 1.7	− 3.4	+ 6.7		
503		182	+ 4.9	+ 4.4	− 6.6	− 1.6		+ 2.7	+ 4.4	+ 4.4		
501		177	+21.6	+ 8.3	+ 8.3	+ 3.8		+ 3.2	+ 1.9	+ 7.6		
756		171									+ 4.6	
755		179									− 7.4	
757		173									− 2.6	
	Mean (±S.E.)		+ 9.2 (±2.8)	+ 8.9 (±3.1)	+ 5.3 (±3.1)	+ 1.6 (±0.8)		+ 3.1 (±1.4)	+ 3.6 (±1.5)	+ 5.0 (±0.8)	− 1.8 (±3.5)	
759	Control	172	− 0.7	+ 3.9	− 2.6	− 2.6		− 0.7				
760		176	− 4.5	+ 4.5	+ 4.5	+ 3.2		+ 3.9				
758		171	− 5.0	+ 6.8	− 0.6	+ 3.1		− 3.1				
	Mean (±S.E.)	177 (±2)	− 3.4 (±1.4)	+ 5.1 (±0.8)	+ 0.4 (±2.1)	+ 1.3 (±1.9)		− 2.6 (±0.9)				

[1] Standard Error of the Mean.
[2] Mg. per 100 ml. whole blood.
* Significant to 5% level. (Obtained by applying "t" test to blood sugar values.)
** Significant to 1% level. (Obtained by applying "t" test to blood sugar values.)

Both the 1 and 5 mg./kg. dosages produced slight hyperglycemia in a majority of the animals, but the mean values for the series were not statistically significant. All of the birds receiving 5 mg./kg. developed lasting hypoglycemia and died in convulsion. None of the birds receiving 1 mg./kg. ever developed hypoglycemia. Blood sugar was determined on three of the birds (Nos. 349, 347, and 348) six days after treatment, and found to be within 3 %of its initial level.

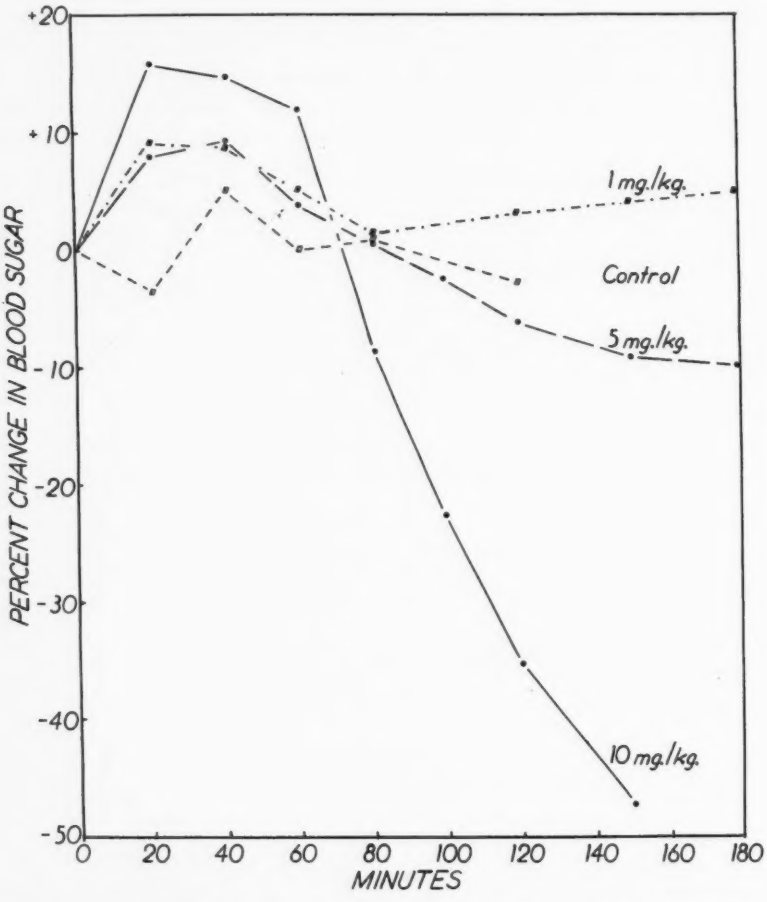


FIG. 1. Blood sugar changes in the fowl following subcutaneous injection of Synthalin A at time 0. Points represent mean values.

Histological Studies

Islets of Langerhans of the fowl may be classified primarily as either alpha islets or beta islets. The beta islets are relatively smaller than the alpha islets, and are found distributed throughout the body of the gland, while the larger alpha islets are generally confined to the proximal portion (11). There is occasional intermingling of cell types within a single Islet mass.

The fine, densely packed cytoplasmic granules of the alpha cells stain red and the nuclei purple with the staining method used. Cell boundaries can be distinguished. The cytoplasmic granules of the beta cells appear larger and less numerous than those of the alpha cells. The beta cell granules stain bluish-green.

Of the ten birds treated with 5 mg. Synthalin A/kg. for histological study, only two lived longer than 18 hours. One began convulsions at 18 hours and the other at 24 hours. Both were killed during convulsions. The pancreases from these two birds were compared histologically with those of control animals.

Some of the alpha cells in both of the treated pancreases appeared to be normally granulated, but most of the cells showed hydropic degranulation. Very rarely was an alpha islet cell of the bird killed 18 hours after treatment completely devoid of cytoplasmic granules. The 24-hour bird, on the other hand, showed complete cytoplasmic degranulation in at least one fourth of the affected cells. In some others, fine strands of granules were observed radiating from the nucleus to the cell membrane through otherwise clear cytoplasm. These strands of granules were not seen in the 18-hour bird. Similar radiating strands were first noted by Davis (1) in the rabbit. Pycnotic cells were rare, and occurred with no greater frequency than in the control islets. The nuclei of the alpha cells of both treated birds were indistinguishable from those of the control alpha cells. No attempt was made in the present study to ascertain the time at which cytological changes in the alpha cells begin to appear.

Except for some thinning-out of cytoplasmic granules, the beta cells of the treated animals appeared unaffected. The acinar cells of the treated birds were indistinguishable from those of the controls.

DISCUSSION

Although the effect of Synthalin A on the pancreatic islet alpha cells appears to be essentially the same in the fowl and the mammals thus far examined, there are some striking differences in blood sugar changes. The maximum temporary hyperglycemia in the bird is reached 20 to 40 minutes after treatment, while that of the rabbit is reached at approximately 4 hours (4). The magnitude of the hyperglycemic phase is much greater in the rabbit than in the chicken. The severe hypoglycemia that follows the transient hyperglycemia leads to convulsions and death in the fowl just as in mammals. The hypoglycemia is always evident in the bird within 2 hours after treatment with 10 mg. Synthalin A/kg. body weight, whereas in the mammal, given a comparable Synthalin A dose, hypoglycemia does not appear until 4 to 16 hours have elapsed (1, 5).

The importance of glucagon in the hormonal regulation of carbohydrate metabolism has not yet been established in birds. Evidence that glucagon is operating in the duck has recently been reported (12, 13).

SUMMARY

The cytotoxic action of Synthalin A on the pancreatic alpha islet cells is demonstrated in the fowl. The cytological changes are comparable to those reported for mammals.

The diphasic change in blood sugar level noted for the mammal is shown to be present in the fowl following subcutaneous injection of Synthalin A. Noteworthy differences, however, appear. Both phases appear more rapidly, and the transient hyperglycemia is far less severe than that reported for mammals.

BRUCE E. BEEKMAN

Eigenmann Fellow in Zoology 1956–57
Zoology Department, Indiana University
Bloomington, Indiana

REFERENCES

1. DAVIS, J. C.: *J. Path. & Bact.* **64**: 575. 1952.
2. RUNGE, W.: *Klin. Wchnschr.* **32**: 748. 1954.
3. VONHOLT, C., AND H. FERNER: *Zschr. Zellforsch.* **42**: 305. 1955.
4. FODDEN, J. H.: *Am. J. Clin. Path.* **23**: 1002. 1953.
5. VONHOLT, C., L. VONHOLT, B. KRONER AND J. KUHNAU: *Arch. exper. Path. u. Pharmakol.* **224**: 66. 1955.
6. OPDYKE, D. F.: *Endocrinology* **31**: 363. 1942.
7. HORVATH, S. M. AND C. A. KNEHR: *J. Biol. Chem.* **140**: 869. 1941.
8. GOMORI, G.: *Am. J. Path.* **17**: 395. 1941.
9. STURKIE, P. C.: *Avian Physiology*, Comstock Publishing Associates, Ithica, N. Y. 1954.
10. SNEDECOR, G.: *Statistical Methods*, 4th Ed., Iowa State College Press, Ames, Iowa. 1953.
11. OAKBERG, E. F.: *Am. J. Anat.* **84**: 279. 1949.
12. MIALHE, P.: *C. rend. Acad. sc.* **241**: 1500. 1955.
13. MIALHE, P.: *C. rend. Acad. sc.* **241**: 1851. 1955.

OBSERVATIONS ON *IN VITRO* ADRENAL STEROID SYNTHESIS IN THE ALBINO MOUSE[1]

The adrenocortical secretions of the rabbit (1, 2) and the laboratory rat (1, 3, 4) are noteworthy in that they usually contain appreciable quantities of corticosterone but no readily detectable amounts of 17α-hydroxycorticosterone (cortisol). The peripheral blood of another rodent, the guinea pig, on the other hand, appears to contain high levels of 17-hydroxycorticosteroids (5).

The impaired ability of the adrenal cortices of certain rodents to hydroxylate 21-carbon steroids at carbon 17 poses an interesting problem in comparative biochemistry. A study has been undertaken to determine to what extent this phenomenon can be detected in other species of rodents. Data on the albino mouse are reported in this communication.

MATERIALS AND METHODS

Adult, female albino mice of the Webster Swiss strain were employed in groups of 40 to 50 animals per experiment. Each incubation flask received the bisected adrenal glands of from 8 to 10 mice, with each mouse contributing, on the average, 6.6 mg. of tissue. The flasks, containing an artificial medium and an atmosphere of pure oxygen, were incubated at 37° C for two and one-half hours.

Ethyl acetate extracts of the contents of the incubation flasks were chromatographed on silica gel columns, an appropriate solvent system being used to obtain a fraction containing the adrenocortical steroids. The steroids so obtained were then merged into one combined extract which was next analyzed for its content of formaldehydogenic substances (FS) and Porter-Silber chromogens (PSC), the latter method being performed in accordance with the authors' instructions (6). The details of the procedures described to this point have been published previously (3).

Prior fractionation on silica gel columns was omitted when the ethyl acetate extracts were to be examined by means of paper chromatography. In this case, the extracts were taken to dryness; the residue was taken up in methanol and put on 42 cm.-long strips of Whatman No. 1 paper. Kendall's Compounds B, E and F were employed routinely as reference standards on one limb of the chromatograms. The chromatograms were first placed

Received July 12, 1956.

[1] Supported by Grant C-2121 from the National Institutes of Health, Public Health Service.

in a hexane tank and overrun with this solvent for from 18 to 24 hours. This removed a large part of the ethyl acetate and methanol-soluble yellowish material which occurred in the extracts and which could not be eliminated by prior chromatography on silica gel columns. Following this depurative procedure, the chloroform-formamide system of Zaffaroni (7) was employed to separate the individual steroids. The running time in this system at 23° C was approximately two hours. The reactive areas on the paper chromatograms were detected by their absorption at 240 mμ and their effect on blue tetrazolium.

RESULTS

1. *Steroidogenesis from endogenous substrates*

The average content of formaldehydogenic substances (FS) in the flasks both before and after the incubation period are reported in Table 1. The rate of formation of FS from endogenous substrates in the two and one-half hours of incubation can thus be determined and is so indicated in the table.

TABLE 1. *In vitro* ADRENOCORTICAL STEROID FORMATION FROM ENDOGENOUS SUBSTRATES

Treatment	No. mice	No. det'ns.	Formaldehydogenic substances	
			μg.[1] / gm. adrenals	Net output[2] μg. / gm. adrenals
Unincubated	90	2	34	—
Incubated	171	4	206	172

[1] Average values expressed as weight equivalents of 11-desoxycorticosterone.
[2] Per two and one-half hours.

Porter-Silber chromogens (PSC) could be detected in only one of the four incubation experiments. The content of the flasks on this occasion was 40 μg. of PSC per gm. of adrenals. The presence of this small quantity of PSC on this one occasion is not readily explicable. It would appear that, under the conditions which obtained in this study, the adrenal cortex of the albino mouse usually does not produce appreciable quantities of adrenocortical steroids possessing a dihydroxyacetone-type side chain.

2. *Tentative identification of the steroids formed under* in vitro *conditions*

To identify, in a tentative fashion the adrenocortical steroids produced in the incubation period, ethyl acetate extracts of the incubation flasks were analyzed by means of paper chromatography. For this purpose, three experiments, involving a total of 121 mice, were performed. The results obtained are illustrated, in a schematic fashion, in Figure 1.

On the basis of visual criteria, it was found that the greatest quantity of material was contained in spot number 4, followed closely by the material in spot number 5. In terms of mobility, spot number 4 is probably not cortisone but may well be aldosterone. By the same criterion, spot number 5 is probably corticosterone. The fact that these two spots both absorb ultra-violet light at 240 mμ and reduce blue tetrazolium adds some support to these provisional identifications.

The two spots (2 and 3) having a mobility roughly that of cortisol do not reduce blue tetrazolium. They were seen on all three chromatograms but their identities are not known.

DISCUSSION

The rate of formation of formaldehydogenic substances from endogenous substrates by the adrenal cortices of albino mice, under the conditions of this study, was 69 μg./gm.

of adrenals/hr. This agrees well with rates of 70 (3) and 67 (8) reported for the Sprague-Dawley strain of laboratory rat under similar circumstances.

On most occasions, the adrenal cortices of the mice failed to produce readily detectable quantities of Porter-Silber chromogens. If this finding is truly indicative of the nature of the secretory products of the adrenal cortex in the albino mouse, then we have in this rodent a most extreme example of an impaired ability to hydroxylate adrenocortical steroids at carbon 17. This seems to be true also, though apparently to a lesser degree, of the laboratory rat (1, 3, 4), the rabbit (1) and the steer (9).

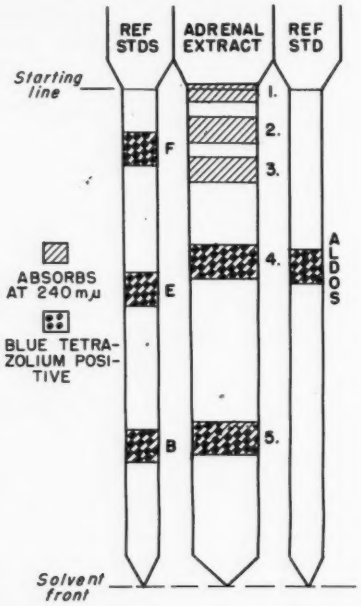


FIG. 1. Schematic representation of the results obtained when the steroids produced *in vitro* by mice adrenal cortices are chromatographed on paper. Reference standards: F (cortisol); E (cortisone); B (corticosterone); and Aldos. (aldosterone).

Because of the limitations of the analytical methods employed in this study, no definitive statements can be made at this time about the identities of the steroids formed by the mouse adrenal cortex under *in vitro* conditions. Tentatively, it can be assumed that spot number 5 (Fig. 1) is corticosterone. As was noted before, the greatest amount of reactive material, by visual criteria, appeared to be in spot number 4. This may be aldosterone or an unresolved mixture of it and other steroids. It most probably is not cortisone. Spot number 4 may well be the counterpart of Fraction 8 of Farrell and Lamus (1) and spot X of Hechter *et al.* (11). A third possibility is that it is one or more compounds, none of which is aldosterone.

As would be expected from the PSC data, no evidence of the secretion of cortisol was found.

SUMMARY

Under the *in vitro* conditions of this study, the adrenal cortices of albino mice produced 69 μg. of formaldehydogenic substances per gram of adrenals per hour. Usually, no readily detectable quantities of Porter-Silber chromogens were formed.

On the basis of paper chromatographic findings, it is suggested that the *in vitro* secretion of the mouse adrenal cortex consists of corticosterone, aldosterone and a number of unidentified compounds but no 17α-hydroxycorticosterone.

The data presented indicate that the albino mouse is an example of those species which have a markedly impaired ability to hydroxylate adrenocortical steroids at carbon 17.

FREDERICK G. HOFMANN[2]

Department of Pharmacology
College of Physicians and Surgeons
Columbia University
New York, New York

Acknowledgments

The author gratefully acknowledges the technical assistance of Miss Flora Hernandez. The aldosterone was kindly furnished by Dr. R. Gaunt of Ciba Pharmaceutical Products, Inc.

REFERENCES

1. BUSH, I. E.: *J. Endocrinol.* **9**: 95. 1953.
2. KASS, E. H., O. HECHTER, I. A. MACCHI AND T. W. MOU: *Proc. Soc. Exper. Biol. & Med.* **85**: 583. 1954.
3. HOFMANN, F. G. AND C. DAVISON: *Endocrinology* **54**: 580. 1954.
4. SINGER, B. AND M. P. STACK-DUNNE: *J. Endocrinol.* **12**: 130. 1955.
5. DONE, A. K., R. S. ELY, L. R. HEISELT AND V. C. KELLEY: *Proc. Soc. Exper. Biol. & Med.* **83**: 722. 1953.
6. PORTER, C. C. AND R. H. SILBER: *J. Biol. Chem.* **185**: 201. 1950.
7. ZAFFARONI, A.: *Recent Prog. Hormone Research.* **8**: 51. 1953.
8. SAFFRAN, M. AND M. J. BAYLISS: *Endocrinology* **52**: 140. 1953.
9. HECHTER, O. AND G. PINCUS: *Physiol. Rev.* **34**: 459. 1954.
10. FARRELL, G. L. AND B. LAMUS: *Proc. Soc. Exper. Biol. & Med.* **84**: 89. 1953.
11. HECHTER, O., I. A. MACCHI, H. KORMAN, E. D. FRANK AND H. A. FRANK: *Am. J. Physiol.* **182**: 29. 1955.

[2] John and Mary R. Markle Scholar in Medical Science.

THE HYPOGLYCEMIC AND INSULINASE-INHIBITORY ACTION OF SOME PLANT GROWTH REGULATORS

The demonstration that the administration of l-tryptophan by mouth to rats results in a decrease in the blood sugar concentration and a concomitant decrease in the activity of insulinase with a consequent decrease in the destruction of insulin (1, 2) led to studies on the effect of various derivatives of l-tryptophan on the action of insulinase *in vitro* and *in vivo*. Such studies revealed that indole-3-acetic acid, among other derivatives of

Aided in part by grants from the Upjohn Company and the Smith, Kline and French Laboratories.

Presented in part before the Pittsburgh Diabetes Association, February 15, 1956.

Received August 8, 1956.

l-tryptophan, not only inhibits the action of insulinase *in vitro* and *in vivo* but also induces a marked hypoglycemia when given to rats by stomach tube (1). The fact that indoleacetic acid is a potent plant growth hormone (auxin) (3) prompted studies on hypoglycemic and insulinase-inhibitory activity of other plant growth regulators.

The present preliminary report deals with the hypoglycemic and insulinase-inhibitory activity of indole-3-acetic acid and two related compounds, indole-3-butyric and indole-3-propionic acids. In addition, to exemplify the action of potent synthetic plant growth regulators, the effect of *p*-chlorphenoxyacetic acid is reported.

METHODS

The indole compounds utilized in the study were dissolved in 0.5% sodium bicarbonate, and the solution was adjusted to pH 8.0 and a concentration which permitted the administration of a dosage of 0.4 mM in 5 ml. per 100 grams of body weight by stomach tube. As controls, the effect of similar concentrations of indole, acetic acid, butyric acid and propionic acid was studied.

After an overnight fast, groups of 10 or more rats of the Carworth strain, weighing more than 120 grams, were given a solution of the plant growth regulator or of 0.5% sodium bicarbonate. Immediately before, and at hourly intervals for five hours after the administration of the solution, blood samples were taken into 0.1 ml. pipettes from the cut tail. The glucose concentration was determined by Nelson's procedure (4).

To determine the *in vivo* insulinase-inhibitory activity of each compound, one group of 10 fed male mice was given a subcutaneous injection of 0.004 mM of the compound in a volume of 0.04 ml. per gram of body weight and a subcutaneous injection of 1 ml. of 5.5% glucose. A second group of 10 mice served as controls and was given a similar volume of 0.5% sodium bicarbonate and glucose. One hour later both groups of mice were given an intraperitoneal injection of 0.4 units of insulin in 0.02 ml. acidified water per gram of body weight and another subcutaneous injection of 1 ml. of 5.5% glucose. The insulin consisted of a mixture of crystalline zinc insulin and tracer quantities of I^{131} labeled insulin. One hour after the intraperitoneal injection of the insulin (i.e., two hours after the subcutaneous injection of the compound) the mice were sacrificed. The preparation of the animals and the determination of the quantity of insulin destroyed per hour per 100 grams of mouse was the same as that described in a previous report (5).

RESULTS AND DISCUSSION

The effect of the three indole compounds on the blood sugar of groups of 10 rats is illustrated in Figure 1. It is apparent that these compounds produce significant depressions in the blood sugar concentration within one hour after the gavage. The hypoglycemic action of indoleacetic acid is more prolonged than that of either indole butyric or indolepropionic acids; the difference between the hypoglycemia induced by indoleacetic acid and that by the other indoles is statistically highly significant by the fifth hour ($P < 0.001$). An even more marked hypoglycemic response follows the oral administration of 4 mM of *p*-chlorphenoxyacetic acid per kilogram to rats (fig. 2).

That the hypoglycemic effect is due to the intact auxin molecule rather than to the indole or the acid in the side-chain is suggested by the fact that a hyperglycemia rather than a hypoglycemia follows the administration of similar concentrations of acetic acid, butyric acid, propionic acid and indole. The relatively rapid restitution of the blood sugar concentration after the administration of indolepropionic acid may be due to the release of indole and propionic acid, which are quite potent as hyperglycemic agents.

The effect of the plant growth hormones on the destruction of insulin by intact mice is summarized in Table 1. In each instance, there was a statistically significant inhibition of the insulinase activity of the animals. It is apparent that like its hypoglycemic action, *p*-chlorphenoxyacetic acid is more potent in inhibiting the destruction of insulin by the intact mouse than are any of the other auxins. Similar results have been obtained in studies on the insulinase activity of rats.

The growth promoting properties of indole-3-acetic acid in plants is well established (6). Likewise, the related compounds, indole-3-butyric and indole-3-propionic acids exhibit auxin activity though to a much smaller degree than indoleacetic acid (7). The

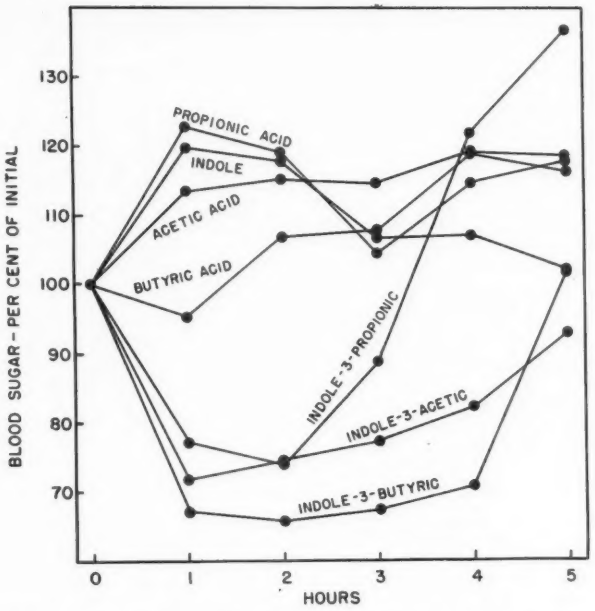


FIG. 1. The effect of indoleacetic, indolepropionic and indolebutyric acids on the blood sugar of rats. The blood sugar concentration at each hourly interval is expressed as the per cent of the initial concentration.

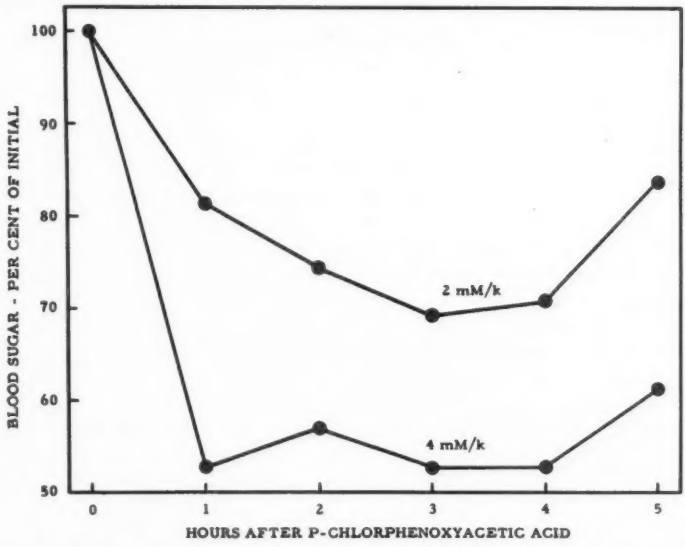


FIG. 2. The effect of *P*-chlorphenoxyacetic acid on the blood sugar of rats. The blood sugar concentration at each hourly interval is expressed as the per cent of the initial concentration.

activity of the synthetic auxin, *p*-chlorphenoxyacetic acid, however, is much greater than that of indoleacetic acid (3). A similar relative potency as hypoglycemic agents is exhibited by these compounds. Whether or not the auxin activity and the hypoglycemic activity of these compounds are related, remains unknown.

Although the mechanism whereby the natural and synthetic plant growth hormones exert their effects in plants is unknown (6), the hypoglycemic response to the ingestion of these compounds appears to be related to a concomitant decrease in insulinase activity.

TABLE 1. EFFECT OF PLANT GROWTH REGULATORS ON DESTRUCTION OF INSULIN BY MICE

Plant growth regulator	Insulin destroyed,* units per 100 grams		Inhibition, per cent
	Sodium bicarbonate	Auxin	
Indoleacetic Acid	10.9±0.7	8.3±0.5	23.9
Indolepropionic Acid	15.1±0.4	12.7±0.6	15.9
Indolebutyric Acid	14.1±0.3	4.7±0.5	66.7
p-chlorphenoxyacetic Acid	12.4±0.8	6.6±0.4	46.8

* Means ±S.E.

Accordingly, the hypoglycemic response may be due to an increase in the availability of endogenous insulin consequent to a decrease in the rate of insulin destruction.

The data reported herein are typical of 16 synthetic auxins that have been studied. Consequently, it appears that the various compounds that have auxin activity are also effective in the inhibition of insulinase and the production of hypoglycemia. The relation of specific chemical constitution to the physiological response in animals will be discussed in a more detailed report. At the present time, however, it is pertinent to note that these studies reveal an hitherto unsuspected relationship between plant and animal hormones.

I. ARTHUR MIRSKY, DANIEL DIENGOTT AND GLADYS PERISUTTI
Department of Clinical Science
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania

REFERENCES

1. MIRSKY, I. A., G. PERISUTTI AND D. DIENGOTT: *Abstract Amer. Chem. Soc.* April, 1956.
2. MIRSKY, I. A., G. PERISUTTI AND D. DIENGOTT: *Endocrinology* (in press).
3. THIMANN, K. V.: in The Hormones **1**: 5. 1948. G. PINCUS AND K. V. THIMANN, New York, 1948.
4. NELSON, N.: *J. Biol. Chem.* **153**: 375. 1944.
5. MIRSKY, I. A., G. PERISUTTI AND R. JINKS: *Endocrinology* **56**: 484. 1955.
6. THIMANN, K. V.: in The Hormones **3**: 1. 1955. G. PINCUS AND K. V. THIMANN, New York, 1955.
7. THIMANN, K. V. AND C. L. SCHNEIDER: *Amer. J. Botany* **25**: 270. 1938.

IN VITRO RESPONSE OF KIDNEY TISSUE METABOLISM TO THYROXINE[1]

The *in vitro* effects of thyroxine have been investigated on many different biological materials, ranging from purified enzymes through broken cell systems to "intact cell" preparations such as tissue slices. Many responses have failed of confirmation, as in the case of the reported (1) immediate metabolic effect of tetraiodothyroacetic acid (TETRAc) and 3,5,3′-triiodothyroacetic acid (TRIAc) on kidney cortex slices (cf. 2). Certain others, such as accelerated succinoxidase activity of heart homogenates, seem to be equally responsive to thyroactive and non-thyroactive compounds provided certain structural relationships hold (3). A major obstacle in obtaining consistent isolated tissue responses to thyroxine is undoubtedly the rapid deterioration of most such preparations. Over the course of several years, this laboratory has obtained scattered evidence for *in vitro* metabolic rate responses to thyroxine, primarily by kidney slices, but with such quantitative variability as to make interpretation difficult. Following the suggestion made by Thibault (4), of incubating at 5° C, we have obtained striking and consistent effects of thyroxine on oxygen consumption of kidney slices.

In general, conventional direct Barcroft-Warburg manometry has been carried out on slices of kidney cortex prepared from thyroidectomized rats. An initial run is made for about one hour at 37°, the vessels are capped with Parafilm and stored at 5° C. At intervals of one day, the vessels are opened, flushed with oxygen and the metabolic rate determined again at 37°, following a fifteen-minute preliminary warming. The vessels are heat-sterilized as part of the routine cleaning procedure, buffered solutions with substrate are prepared fresh for each experiment and all procedures, including tissue slicing, weighing, etc., are carried out in a careful manner. However, no extraordinary aseptic precautions have been taken, since a survey of several flasks, kindly performed by Dr. C. H. Winkler of the Department of Microbiology, indicated less than 500 organisms per ml. after incubation.

With the oxygen consumption of the control tissue in Krebs' Ringer phosphate glucose solution being considered 100%, that with 5–10 μg. thyroxine per ml. is seen in Figure 1 to be 43% greater after 1 day, 144% greater by the second day, and 273% on the third. This is not a true stimulation of metabolism, since oxygen consumption of the control tissue fell off rapidly to about 55%, 30% and 15% of the starting value on days 1, 2 and 3, respectively.

Tris-hydroxymethyl-methylamine plus hydrochloric acid to a pH of 7.5 was used as a non-phosphate buffer with Krebs' Ringer glucose, but was found far inferior to the phosphate system in maintenance of respiration over 1–2 days. When a glycine-NaOH buffer was substituted for the phosphate, control respiration was the same. However, as the figure shows, addition of thyroxine was able to maintain activity as high as the starting level for 24 hours. Even at 48 and 72 hours, respiration had fallen only 15 and 30%, respectively. Further trials revealed that this was more a specific amino acid effect than a buffering one, since glycyl-glycine and glycyl-glycyl-glycine were far less effective, even with thyroxine present, than glycine itself. Figure 1 includes the results for the dipeptide.

Of several other amino acids tried, DL-alanine has been found to offer the most advantageous conditions for long-term incubation. As that portion of the figure demonstrates, even the control flasks remained near the initial oxygen consumption level for the first 24 hours, and with thyroxine present, the kidney slice metabolism in three

---

Received August 16, 1956.

[1] This work was supported by grants from the American Cancer Society and from the Smith, Kline and French Foundation. Thyroxine was supplied by Dr. H. A. Fevold, of the Travenol Division of Baxter Laboratories.

experiments averaged 22% and 14% above the initial at 24 and 48 hours of incubation, respectively.

Paper chromatography of the incubation medium has revealed that small amounts of phenylalanine, leucine, glutamic acid, lysine and serine are present in the incubation media after incubation, presumably originating in the tissue. The variety of amino acids does not seem to depend upon the particular amino acid present in the original fluid. The presence or absence of thyroxine has no prominent influence upon the nature or quantity of the amino acids. Chromatography of methanol-ammonia extracts of

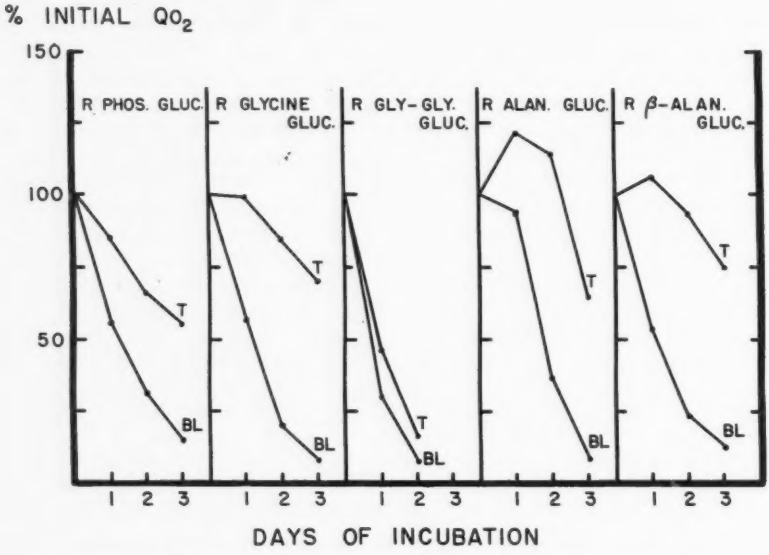


FIG. 1. Changes in oxygen consumption of thyroidectomized rat kidney slices incubated in the solutions indicated (Ringer's-phosphate-glucose, Ringer's-glycine-glucose, Ringer's-glycylglycine-glucose, Ringer's-alanine-glucose, and Ringer's-β-alanine-glucose). Metabolism determinations were made at 37° C; incubation was at 5° C without shaking. (T = thyroxine added, BL = blank.)

separated incubation medium and tissue strongly suggests that the added thyroxine was completely taken up by the slices. At the end of the incubation period, 3,5,3′-triiodothyronine, and TETRAc have been tentatively identified in tissue extracts, but not in the media.

Undue enthusiam should be avoided in interpreting these results in terms of the *in vivo* response of metabolism to injected thyroxine. A concentration of 5 μg. of thyroxine per ml. incubation fluid is about one hundred times the physiological level of the hormone in the blood plasma or in kidney tissue of euthyroid animals. The incubation results actually appear to be distinctly better on the third day if the amount of hormone present is raised to 10 μg. per ml., although some effect is seen at 1 μg. per ml. On the other hand, the phenomenon does not appear until about 6 to 12 hours of incubation, even though this time is spent at 5° C. It does appear possible that some insight into thyroxine action may be gained from further study of this *in vitro* response.

SUMMARY

In vitro effects of thyroxine on the oxygen consumption of kidney cortex slices in long-term incubation experiments were studied by direct Barcroft-Warburg manometry. Of a number of amino acids included in the incubation medium, DL-alanine has been found to offer the most advantageous conditions for maintaining oxygen consumption.

S. B. BARKER

Department of Pharmacology
University of Alabama Medical Center,
Birmingham, Alabama

REFERENCES

1. THIBAULT, O. AND R. PITT-RIVERS: *Compt. rend. soc. biol.* **149**: 880. 1955.
2. BARKER, S. B. AND W. J. LEWIS: *Proc. Exper. Biol. and Med.* **91**: 650. 1956.
3. BARKER, S. B. AND W. J. LEWIS: *Fed. Proc.* **15**: 8, 1956.
4. THIBAULT, O.: *Compt. rend. soc. biol.* **149**: 877. 1955.

ENDOCRINOLOGY STUDY SECTION NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MARYLAND

The Endocrinology Study Section of the National Institutes of Health, Bethesda 14, Maryland, announces a plan for supplying anterior pituitary hormones, other than ACTH, to qualified investigators in the medical sciences. Five of the individual Institutes of the National Institutes of Health are jointly providing the funds for the purchase or production of large uniform lots of the hormones, purified to meet exacting specifications, set by the Study Section, both for potency and for low limits of contamination with other activities.

The first lots of bovine growth hormone and of ovine prolactin have been approved by the Study Section and will be ready for distribution about November 1st. 1956, The growth hormone and prolactin are packaged as sterile, lyophilized powders in vials of 50 mg. and 25 mg. respectively. Data on the estimated potency and degree of contamination, and instructions for dissolving the materials, will be issued with each package. A pilot plant at Emory University is being set up under the direction of Drs. Stanley Ellis and Alfred E. Wilhelmi for the production of follicle-stimulating, luteinizing and thyrotrophic hormones. These materials will not be ready for some months.

Details of the program are being looked after by a Subcommittee of present and past members of the Study Section: Drs. Warren O. Nelson, Roy Hertz, Robert W. Bates, and Alfred E. Wilhelmi, Chairman. Dr. Gregory Pincus is Chairman of the Study Section and Dr. Sam R. Hall is its Executive Secretary. In Addition, the Subcommittee enjoys the expert advice and services of Drs. W. H. McShan, George Sayers, Paul Munson, C. H. Li, John G. Pierce, and Peter Condliffe, who act as consultants.

Grants of the hormones will be made to qualified investigators who apply to the Endocrinology Study Section. Application may be made by a letter setting forth a brief description of the proposed work, together with an estimate of the amount of hormones required. Requests will be granted to the extent that supplies of the hormones allow.

It is hoped that by this service both clinical and fundamental studies on the anterior pituitary hormones will be stimulated. At the same time, it is thought that much advantage will be gained from the fact that a large number and variety of studies may be made by numerous investigators all using the same highly purified and carefully tested preparations. Continuing support for the program will be sought if, as is thought by the Study Section, work in these fields has been limited by the availability of suitable materials.

AMERICAN CANCER SOCIETY

The American Cancer Society announced today that it is prepared now to receive applications for research grants under its newly reorganized research program.

Application forms may be obtained by writing to the American Cancer Society, 521 West 57th Street, New York 19, N. Y. Applications received before January First will be acted upon during the late winter and early spring, and most grants will become effective July 1, 1957, Other deadlines for receipt of applications will be May 1 and September 1.

Types of grants include project grants, program grants, institutional research grants, contracts for research, post-doctoral fellowships, scholars in cancer research and additional faculty-level positions.

INTERNATIONAL ABSTRACTS OF BIOLOGICAL SCIENCES

Notice of Change of Title and Coverage

The Council of Biological and Medical Abstracts Ltd. has decided to change the title of their journal *British Abstracts of Medical Sciences* to

International Abstracts of Biological Sciences

This decision results from two major developments. The first is an extension of coverage to a wider range of biological research subjects published in journals throughout the world, and the second is that the International Abstracts of Biological Sciences, with the co-operation of the specialist editors selected by the Institute of Scientific Information of the Academy of Sciences of the U.S.S.R. will include, as from the January 1957 issue, translations of the important Russian papers abstracted in the *Referativny Zhurnal Biologii* (Soviet Biological Abstracts) and *Referativny Zhurnal Biologischekoi Khimii* (Soviet Abstracts of Biological Chemistry), to be published simultaneously with their appearance in Russian.

International Abstracts of Biological Sciences abstracts the world literature in the following fields: Anatomy, Animal Behaviour, Biochemistry, Biophysics, Cytology, Embryology, Endocrinology, Epidemiology, Experimental Biology, Genetics, Haematology, Histochemistry, Histology, Immunology, Microbiology, Nutrition, Odontology, Parasitology, Pathology, Pharmacology, Physical Anthropology, Physiology, Radiation Effects, Toxicology and Viruses.

International Abstracts of Biological Sciences is published for the Council of Biological and Medical Abstracts Ltd. by Pergamon Press, London and New York, on a non profit-making basis.

AUTHOR INDEX, VOLUME 59

ADROUNDY, GEORGE A. AND JANE A. RUSSELL. Growth Hormone and Nutritional Status on Cardiac Glycogen in the Rat, 241

ALBRIGHT, EDWIN C., F. C. LARSON, KENKICHI TOMITA AND HENRY A. LARDY. Enzymatic Conversion of Thyroxine and Triiodothyronine to the Corresponding Acetic Acid Analogues, 252

ALLEGRETTI, NIKŠA, MLADEN EST AND DURDA URAIĆ. Alloxan Administration in the Guinea Pig with Acinous Tissue Rendered Atrophic by Ligature of the Pancreatic Duct, 131

ANTHONY, PAUL Z., see TABACHNICK, IRVING I., 153

ANTLIFF, HAROLD R. AND WILLIAM C. YOUNG. Behavioral and Tissue Responses of Male Guinea Pigs to Estrogens and the Problem of Hormone Specificity, 74

BAKER, BURTON L., J. H. KLEGMAN, R. G. LOGAN AND R. L. GARNER. Reduction in Parotid Amylase after Hypophysectomy, 254

BAKER, C., see SWINGLE, W. W., 226

BAKHTIAR, ABOLFATH KHAN, see BISCHOFF, FRITZ, 138

BANKS, J., see ORTIZ, E., 479

BARKER, S. B., Metabolic Actions of Thyroxine Derivatives and Analogs, 548

BARKER, S. B., *In Vitro* Response of Kidney Tissue Metabolism to Thyroxine, 719

BARNUM, C. P., see HALBERG, F. H., 364

BARRNETT, RUSSELL, J., AARON J. LADMAN, NANCY J. MCALLASTER AND ELEANOR R. SIPERSTEIN. The Localization of Glycoprotein Hormones in the Anterior Pituitary Glands of Rats Investigated by Differential Protein Solubilities, Histolog-Stains and Bioassays, 398

BASCOM, WILLARD D., see ROSENFELD, GEORGE, 497

BATTS, ADRIENNE A., LESLIE L. BENNETT, STANLEY ELLIS AND ROBERT A. GEORGE. Growth Hormone-induced Reduction of Glycosuria and Partial Repair of the Islets of Langerhans in Partially Pancreatectomized Diabetic Rats, 620

BEATON, GEORGE H. A Comparison of Two Growth Hormone Preparations, 356

BEEKMAN, BRUCE E. The Effect of Synthalin A on Blood Sugar and Pancreatic Alpha Islet Cells of the Fowl, 708

BENNETT, LESLIE L., see BATTS, ADRIENNE A., 620

BERMAN, IRWIN, ABRAHAN EDELMANN AND ALBERT S. GORDON. The Relation of the Adrenal to Phosphorus Metabolism of Bone Marrow, 656

BERRY, ROGER J., see GRUNT, JEROME A., 540

BEYER, ARLEY T., see VELARDO, JOSEPH T., 165

BIRMINGHAM, MARION K., ERIKA KURLENTS, G. J. ROCHEFORT, M. SAFFRAN AND A. V. SCHALLY. ACTH Content of Rat Pituitary Glands, 677

BISCHOFF, FRITZ AND ABOLFATH KHAN BAKHTIAR. Shaw Adrenaline Test, 138

BITTNER, J. J., see HALBERG, F., 380

BJORN, JOHN C. AND W. U. GARDNER. Inguinal Hernias in Female Mice Treated with Androgens or Bearing Grafts of Testes, 48

BLACKMORE, WILLIAM P. AND HILDA T. CHESTER. Assay of Antidiuretic Substance in Plasma of Normal and Diabetes Insipidus Dogs, 493

BOGDANOVE, E. M., see ESKIN, B. A., 688

BOIS, PIERRE, see SELYE, HANS, 646

BOOKER, WALTER M., FRANCES DACOSTA, SAMUEL Q. MITCHELL AND MARVIN SKELTON. The Relation of Succinate to Adrenocortical Function, 317

BRANNICK, L. J., see SWINGLE, W. W., 226

BRIGGS, NORMAN F., see WELLS, HERBERT, 571

BRODISH, A. AND C. N. H. LONG. Changes in Blood ACTH under Various Experimental Conditions Studies by

Means of a Cross-circulation Technique, 666
Brown, J., see Gardocki, J. F., 129
Browning, K. M., see Gemmill, C. L., 522
Burgos, Mario H. and George B. Wislocki. The Cyclical Changes in the Guinea Pig's Uterus, Cervix, Vagina and Sexual Skin, Investigated by Histological and Histochemical Means, 93

Calhoun, David W., see Edgren, Richard A., 631
Carlson, L. D., see Cottle, Merva, 1
Carlson, L. D., see Woods, Richard, 323
Carnes, William H., see Kriss, Joseph P., 555
Chaikoff, I. L., see Potter, G. D., 12
Chaikoff, I. L., see Taurog, Alvin, 34
Chang, M. C., see Pincus, Gregory, 695
Chester, Hilda T., see Blackmore, William P., 493
Clark, Patricia G., see Shafer, William G., 516
Cottle, Merva and L. D. Carlson. Turnover of Thyroid Hormone in Cold-Exposed Rats Determined by Radioactive Iodine Studies, 1
Courtright, Claiborne L., see Shetlar, M. R., 532
Crafts, Roger C., see Meineke, Howard A., 444
Crispell, K. R., see Gemmill, C. L., 522

DaCosta, Frances, see Booker, Walter M., 317
D'Angelo, Savino A. and Ronald E. Traum. Pituitary-Thyroid Function in Rats with Hypothalamic Lesions, 593
Danowski, T. S., see Feldman, Joseph D., 463
Davis, James S., Roland K. Meyer and W. H. McShan. The Effects of Aminopterin and Estrogen on the Phosphate Metabolism of Rat Uterus, 505
Diengott, Daniel, see Mirsky, I. Arthur, 369
Diengott, Daniel, see Mirsky, I. Arthur, 715
Dimick, B. S., see Mayer, S. W., 586
Durda, Uraić, see Allegretti, Nikŝa, 131

Edelmann, Abraham, see Sherwood M. Reichard, 55
Edelmann, Abraham, see Berman, Irwin, 656
Edgren, Richard A. and David W. Calhoun. Density as an Index of the Effects of Estrogens on Bone, 631
Elliott, J. R., see Talmage, Roy V., 27
Elliott, Joseph R. and Smith Freeman. Parathyroid Function and the Plasma Citric Acid and Calcium Response, 181
Elliott, Joseph R., see Freeman, Smith, 190
Elliott, Joseph R. and Smith Freeman, Relative Effect of Vitamin D and Parathyroid Extract on Plasma Calcium and Citric Acid of Normal and Thyroparathyroidectomized Dogs, 196
Ellis, Stanley, see Batts, Adrienne A., 620
Ely, Charles A. Effect of Antigonadotrophic Serum on Recent Intrasplenic Ovarian Implants of Castrate Mice, 83
Engel, Lewis L., see Ryan, Kenneth J., 499
Eskin, B. A. and E. M. Bogdanove. The Influence of Estrogen upon Goiter Induction in Adult and Immature Rats, 688
Est, Mladen, see Allegretti, Nikŝa, 131
Everett, Jown W. The Time of Release of Ovulating Hormone from the Rat Hypophysis, 580

Farrell, Gordon, see Fleming, Ronald, 360
Farrell, Gordon L., see Rauschkolb, Elizabeth W., 526
Feldman, Joseph D. Effect of Estrogen on Thyroidal Iodide Trapping and Conversion of Inorganic I^{131} to Protein Bound I^{131}, 289
Feldman, Joseph D. and T. S. Danowski. Effect of Estrogen on the Metabolism of Protein-Bound Iodine, 463
Firmat, George, see Roberts, Kathleen E., 565
Fleming, Harold S. Growth and Development of Tooth Germ Homotransplants in Female Guinea Pig Castrates, 119
Fleming, Ronald and Gordon Farrell. Aldosterone and Hydrocortisone Secretion by the Denervated Adrenal, 360
Flores, Albert, see Segaloff, Albert, 233

FREEMAN, SMITH, see ELLIOTT, JOSEPH R., 181

FREEMAN, SMITH AND JOSEPH R. ELLIOTT. The Effect of Fluoroacetate upon the Plasma Citrate Response to Parathyroidectomy and Nephrectomy, 190

FREEMAN, SMITH, see ELLIOTT, JOSEPH R., 196

FRIEDEN, EDWARD H. The Effects of Estrogen and Relaxin Upon the Uptake of Glycine-1-C^{14} by Connective Tissue *In Vivo*, 69

FRITZ, I. B., see TAUBENHAUS, M., 458

GANONG, WILLIAM F. AND DAVID M. HUME. The Effect of Graded Hypophysectomy on Thyroid, Gonadal, and Adrenocortical Function in the Dog, 293

GANONG, WILLIAM F. AND DAVID M. HUME. The Effect of Unilateral Adrenalectomy on Adrenal Venous 17-Hydroxycorticosteroid Output in the Dog, 302

GARDNER, W. U., see BJORN, JOHN C., 48

GARDOCKI, J. F., S. Y. P'AN AND J. BROWN. Lack of Biological Activities of 21-Hydroxypregnane-3, 20-Dione Sodium Succinate, 129

GARNER, R. L., see BAKER, BURTON L., 254

GEMMILL, C. L., K. R. CRISPELL AND K. M. BROWNING. The Basal Metabolism of Rats Following the Injection of Radioactive Iodide, 522

GEMZELL, CARL A. AND FRANK HEIJKENSKJÖLD. Growth Hormone Content in Human Pituitaries, 681

GEORGE, ROBERT A., see BATTS, ADRIENNE, A., 620

GORBMAN, AUBREY, see SHELLABARGER, C. J., 331

GORDON, ALBERT S., see REICHARD, SHERWOOD M., 55

GORDON, ALBERT S., see BERMAN, IRWIN, 656

GREENSPAN, FRANCIS S., see KRISS, JOSEPH P., 555

GRUNT, JEROME A., WILLIAM H. KNISELY AND ROGER J. BERRY. *In Vivo* Observations on the Seminal Vesicles of Intact, Castrated and Hormonally Treated Rats, 540

GUILLEBEAU, JAMES, see SKELTON, FLOYD R., 201

HAFEZ, E. S. E., see PINCUS, GREGORY, 695

HALBERG, E., see HALBERG, F., 364

HALBERG, F., H. VERMUND, E. HALBERG AND C. P. BARNUM. Adrenal Hormones and Phospholipid Metabolism in Liver Cytoplasm of Adrenalectomized Mice, 364

HALBERG, F., W. W. SPINK AND J. J. BITTNER. Protection by Aldosterone and 11,17,-Oxycorticoids against Effects of Brucella Somatic Antigen in Adrenalectomized Mice, 380

HALMI, N. S. AND R. G. STUELKE. Radioiodine Not Dischargeable with Perchlorate in the Thyroids of Propylthiouracil Treated Rats, 134

HARRIS, G. W., see TAUROG, ALVIN, 34

HEIJKENSKJÖLD, FRANK, see GEMZELL, CARL A., 681

HELLBAUM, A. A., see PAYNE, R. W., 306

HIRSCHOWITZ, BASIL I., DAVID H. P. STREETEN AND H. MARVIN POLLARD. Influence of Adrenal Cortex on Gastric Pepsinogen in the Rat, 419

HISAW, FREDERICK L., see VELARDO, JOSEPH T., 165

HOFMANN, FREDERICK G. Observations on *In Vitro* Adrenal Steroid Synthesis in the Albino Mouse, 712

HOUSE, E. L., P. F. NACE AND J. P. TASSONI. Alloxan Diabetes in the Hamster: Organ Changes During the First Day, 433

HOUSSAY, B. A. AND J. C. PENHOS. Diabetogenic Action of Pituitary Hormones on Adrenalectomized-Hypophysectomized Dogs, 637

HOWARD, PALMER R., see SHETLAR, M. R., 532

HUME, DAVID M., see GANONG, WILLIAM F., 293

HUME, DAVID M., see GANONG, WILLIAM F., 302

INGLE, DWIGHT J. Urinary Glucose and Tumor Growth in Diabetic Rats, 259

JOEL, WALTER, see SHETLAR, M. R., 532

JÖRGENSEN, C. BARKER, K. G. WINGSTRAND AND PER ROSENKILDE. Neurohypophysis and Water Metabolism in the Toad, *Bufo Bufo* (L), 601

JOUAN, PIERRE, see ROCHE, JEAN, 425

KELLY, F. H., see MAYER, S. W., 586

KELLY, THOMAS L., see STEELMAN, SANFORD L., 256

KLEGMAN, J. H., see BAKER BURTON L., 254

KNISELY, WILLIAM H., see GRUNT, JEROME A., 540
KOBAYASHI, HIDESHI, see MARUYAMA, KOSÇAK, 213
KOCSIS, J. J. Uptake of P^{32} into Various Phosphorus-Containing Fractions in the Brain of Normal, Hypophysectomized and ACTH-Treated Hypophysectomized Rats, 591
KRISS, JOSEPH P., FRANCIS S. GREENSPAN, WILLIAM H. CARNES AND WILLIAM LEW. Alterations in Chick Thyroid Function Induced by Cobalt, 555
KURLENTS, ERIKA, see BIRMINGHAM, MARION K., 677

LADMAN, AARON J., see BARRNETT, RUSSELL J., 398
LARDY, HENRY A., see ALBRIGHT, EDWIN C., 252
LARSON, F. C., see ALBRIGHT, EDWIN C., 252
LATTA, M. J., see MCDONALD, D. F., 159
LAUFER, HANS, see ROMANOFF, ALEXIS L., 611
LEBLOND, C. P., see VULPÉ, M., 257
LEBRIE, STEPHEN, see SWINGLE, W. W., 226
LEHR, DAVID AND CONSTANCE R. MARTIN. Prevention of Severe Cardiovascular and Smooth Muscle Necrosis in the Rat by Thyroparathyroidectomy, 273
LEW, WILLIAM, see KRISS, JOSEPH P., 555
LOGAN, R. G., see BAKER, BURTON L., 254
LONG, C. N. H., see ULRICH, FRANK, 170
LONG, C. N. H., see BRODISH, A., 666

MARTIN, CONSTANCE R., see LEHR, DAVID, 273
MARUYAMA, KOSÇAK AND HIDESHI KOBAYASHI. Effect of Thyroxine on Latent Apyrase Activity of Canary Liver Mitochondria, 213
MAYER, S. W., B. W. DIMICK AND F. H. KELLY. Further Observations on the I^{131} Metabolism of Pre-Frozen Thyroid Slices, 586
MCALLASTER, NANCY J., see BARRNETT, RUSSELL J., 398
MCDONALD, D. F. AND M. J. LATTA. Anerobic Glycolysis of Human Prostatic Adenoma: *In Vitro* Inhibition by Estrogen and by Androgen and Estrogen, 159
MCGILL, D., see SHELLABARGER, C. J., 331
MCSHAN, W. H., see DAVIS, JAMES S., 505
MEINEKE, HOWARD A. AND ROGER C. CRAFTS. The Effect of Growth Hormone on Hematopoiesis in Hypophysectomized Adult Female Rats, 444
MERRILL, ANNE, see PINCUS, GREGORY, 695
MEYER, ROLAND K., see DAVIS, JAMES, S., 505
MICHEL, RAYMOND, see ROCHE, JEAN, 425
MIRSKY, I. ARTHUR, GLADYS PERISUTTI AND DANIEL DIENGOTT. The Hypoglycemic and Insulinase Inhibitory Action of L-Tryptophan, 369
MIRSKY, I. ARTHUR, DANIEL DIENGOTT AND GLADYS PERISUITTI, 715
MITCHELL, SAMUEL Q., see BOOKER, WALTER M., 317
MORTON, J. V., see TAUBENHAUS, M., 458
MUHLER, JOSEPH C., see SHAFER, WILLIAM G., 516
MUNSON, PAUL L., see WELLS, HERBERT, 571

NACE, P. F., see HOUSE, E. L., 433
NELSON, WARREN O., Endocrinology—Achievement and Challenge. 1956 Presidential Address, 140

OHLER, E. A. AND R. W. SEVY. Inhibition of Stress Induced Adrenal Ascorbic Acid Depletion by Morphine, Dibenzyline and Adrenal Cortex Extract, 347
OWENS, J. N., JR., see PAYNE, R. W., 306
ORTIZ, E., D. PRICE, H. G. WILLIAMS-ASHMAN AND J. BANKS. The Influence of Androgen on the Male Accessory Reproductive Glands of The Guinea Pig: Studies on Growth, Histological Structure and Fructose and Citric Acid Secretion, 479

P'AN, S. Y., see GARDOCKI, J. F., 129
PARLOW, A. F., see SWINGLE, W. W., 226
PARKER, R. E., see TABACHNICK, IRVING I., 153
PAYNE, R. W., A. A. HELLBAUM AND J. N. OWENS, JR. The Effect of Androgens on the Ovaries and Uterus of the Estrogen Treated Hypophysectomized Immature Rat., 306
PENHOS, J. C., see HOUSSAY, B. A., 637
PERISUITTI, GLADYS, see MIRSKY, I. ARTHUR, 369
PERISUTTI, GLADYS, see MIRSKY, I. ARTHUR, 715
PINCUS, GREGORY, M. C. CHANG, M. X. ZARROW, E. S. E. HAFEZ, AND ANNE

MERRILL. Studies of the Biological Activity of Certain 19—nor Steroids in Female Animals, 695

POLLARD, MARVIN H., see HIRSCHOWITZ, BASIL I., 419

POTTER, G. D., ALVIN TAUROG AND I. L. CHAIKOFF. The I^{131}-Irradiated Rat Thyroid: Its Altered Response to Various Stimuli and the Changes Induced in Its Iodine Metabolism, 12

PRICE, D., see ORTIZ, E., 479

PRUNIER, JOHN, see ROBERTS, KATHLEEN E., 565

RAUSCHKOLB, ELIZABETH W., AND GORDON L. FARRELL. Evidence for Diencephalic Regulation of Aldosterone Secretion, 526

RAWSON, RULON W., see ROBERTS, KATHLEEN E., 565

REICHARD, SHERWOOD M., ABRAHAM EDELMANN AND ALBERT S. GORDON. Adrenal and Hypophyseal Influences Upon the Uptake of Radioactive Gold (Au^{198}) by the Reticulo-Endothelial System, 55

REIFENSTEIN, EDWARD C., JR., see SHETLAR, M. R., 532

RICHTER, CURT P. Loss of Appetite for Alcohol and Alcoholic Beverages Produced in Rats by Treatment with Thyroid Preparations, 472

ROBERTS, KATHLEEN E., GEORGE FIRMAT, JOHN PRUNIER, MORTON K. SCHWARTZ AND RULON W. RAWSON. Effect of Phosphate in Enhancing the Action of Triiodothyronine, 565

ROCHE, JEAN, RAYMOND MICHEL, PIERRE JOUAN AND WALTER WOLF. The Recovery of 3:5:3′-Triiodothyroacetic Acid and 3:3′-Diiodothyronine from Rat Kidney After Injection of 3:5:3′-Triiodothyronine, 425

ROCHEFORT, G. J., see BIRMINGHAM, MARION K., 677

ROMANOFF, ALEXIS L. AND HANS LAUFER. The Effect of Injected Thiourea on the Development of Some Organs of the Chick Embryo, 611

ROOT, MARY A. Effect of Glucagon on Glycogen in Rats and Rabbits, 340

ROSENFELD, GEORGE AND WILLARD D. BASCOM. *In Vitro* Studies of the Influence of Corticotropin, Somatotropin, Thyrotropin and Gonadotropin on Adrenal Steroidogenesis, 497

ROSENKILDE, PER, see JORGENSEN, C. BARKER, 601

RUSSELL, JANE A., see ADROUNY, GEORGE A., 241

RYAN, KENNETH J. AND LEWIS L. ENGEL. Estrogen Metabolites in Human Serum, 499

SAFFRAN, M., see BIRMINGHAM, MARION K., 677

SANTISTEBAN, GEORGE A. AND CHESTER A. SWINYARD. The Effect of Ethyl Alcohol on Adrenal Cortical Activity in Mice, 391

SCHALLY, A. V., see BIRMINGHAM, MARION K., 677

SCHATZLEIN, FRANK C., see SHELLABARGER, C. J., 331

SCHEIN, A. H. Is Xanthine Oxidase Present in Mammalian Thyroid Glands?, 596

SCHMIDT, IDA G. An Accessory Adrenal Cortex in the Kidney of a Rhesus Monkey and the Effects of Pyrimethamine on This Tissue, 454

SCHWARTZ, MORTON K., see ROBERTS, KATHLEEN E., 565

SCHWEIZER, MALVINA. An Explanation of Some Apparent Anomalies in the Alteration of Eosinophil Levels in the Guinea Pig, 495

SCHWEIZER, MALVINA. Effect of Testosterone on Circulating Eosinophils in the Guinea Pig, 642

SEGALOFF, ALBERT, SANFORD L. STEELMAN AND ALBERT FLORES. Prolactin as a Factor in the Ventral Prostate Assay for Luteinizing Hormone, 233

SEGALOFF, ALBERT, see STEELMAN, SANFORD L., 256

SELYE, HANS AND PIERRE BOIS. On the Nephrosclerotic Action of 2-Methyl-9-Fluorocortisol, a Highly Active Artificial Mineralocorticoid, 646

SEVY, R. W., see OHLER, E. A., 347

SHAFER, WILLIAM G., PATRICIA G. CLARK AND JOSEPH C. MUHLER. The Inhibition of Hypophysectomy-Induced Changes in the Rat Submaxillary Glands, 516

SHELLABARGER, C. J., AUBREY GORBMAN, FRANK C. SCHATZLEIN AND D. MCGILL. Some Quantitative and Qualitative Aspects of I^{131} Metabolism in Turtles, 331

SHETLAR, M. R., R. PALMER HOWARD, WALTER JOEL, CLAIBORNE L. COURTRIGHT AND EDWARD C. REIFENSTEIN, JR. The Effects of Parathyroid Hormone on Serum Glycoprotein and

Seromucoid Levels and on the Kikney of the Rat, 532

SIPERSTEIN, ELEANOR R., see BARRNETT, RUSSELL J., 398

SKELTON, FLOYD R. AND JAMES GUILLEBEAU. The Influence of Age on Development of Adrenal-Regeneration Hypertension, 201

SKELTON, MARVIN, see BOOKER, WALTER M., 317

SPINK, W. W., see HALBERG, 380

STEELMAN, SANFORD L., see SEGALOFF, ALBERT, 233

STEELMAN, SANFORD L., THOMAS L. KELLY, ALBERT SEGALOFF AND G. F. WEBER. Isolation of an Apparently Homogeneous Follicle Stimulating Hormone, 256

STREETEN, DAVID H. P., see HIRSCHOWITZ, BASIL I., 419

STUELKE, R. G., see HALMI, N. S., 134

SULKOWITCH, HIRSH. The Colorimetric Estimation of the Epinephrines, Dihydroxyindoles and Their Quinones with Aromatic Orthodiamines, 260

SWANSON, HEIDI EARTLY. Interrelations Between Thyroxin and Adrenalin in the Regulation of Oxygen Consumption in the Albino Rat, 217

SWINGLE, W. W., L. J. BRANNICK, A. F. PARLOW, C. BAKER AND STEPHEN LEBRIE. Life Maintenance and Mineralocorticoid Activity of Some New Derivatives of Cortisone Hydrocortisone and DOC, 226

SWINYARD, CHESTER A., see SANTISTEBAN, GEORGE A., 391

TABACHNICK, IRVING I. A., R. E. PARKER, J. WAGNER AND PAUL Z. ANTHONY. A Re-Evaluation of Calorigenic Action of L- and D-Isomers of Thyroxine, 153

TALMAGE, ROY V. AND J. R. ELLIOTT. Changes in Extracellular Fluid Levels of Calcium, Phosphate and Citrate Ions in Nephrectomized Rats Following Parathyroidectomy, 27

TASSONI, J. P., see HOUSE, E. L., 433

TAUBENHAUS, M., I. B. FRITZ AND J. V. MORTON. *In Vitro* Effects of Steroids upon Electrolyte Transfer Through Frog Skin, 458

TAUROG, ALVIN, see POTTER, G. D., 12

TAUROG, ALVIN, G. W. HARRIS, W. TONG, AND I. L. CHAIKOFF. The Uptake of I^{131}-Labeled Thyroxine and Triiodothyronine by the Neurohypophysis, 34

TOMITA, KENKICHI, see ALBRIGHT, EDWIN C., 252

TONG, W., see TAUROG, ALVIN, 34

TRAUM, RONALD E., see D'ANGELO, SAVINO A., 593

ULRICH, FRANK AND C. N. H. LONG. Effects of Stress on Serum C^{14} Levels in Rats Following Administration of Hydrocortisone-4-C^{14} and Corticosterone-4-C^{14}, 170

URAIĆ, DURDA, see ALLEGRETTI, NIKŠA, 131

USHER, D., see VULPÉ, M., 257

VELARDO, JOSEPH T., FREDERICK L. HISAW AND ARLEY T. BEYER. Inhibitory Action of Desoxycorticosterone Acetate, Cortisone Acetate, and Testosterone on Uterine Growth Induced by Estradiol-17β, 165

VERMUND, H., see HALBERG, F., 364

VULPÉ, M., D. USHER AND C. P. LEBLOND. Influence of the Extirpation of Male Accessories on the Occurrence of a "Soft Calculus" in the Urinary Bladder of the Rat, 257

WAGNER, J., see TABACHNICK, IRVING I. A. 153

WEBER, G. F., see STEELMAN, SANFORD L., 256

WELLS, HERBERT, F. NORMAN BRIGGS AND PAUL L. MUNSON. The Inhibitory Effect of Reserpine on ACTH Secretion in Response to Stressful Stimuli, 571

WILLIAMS-ASHMAN, H. G., see ORTIZ, E., 479

WINGSTRAND, K. G., see JORGENSEN, C. BARKER, 601

WISLOCKI, GEORGE B., see BURGOS, MARIO H., 93

WOODS, RICHARD AND L. D. CARLSON. Thyroxine Secretion in Rats Exposed to Cold, 323

WOLF, WALTER, see ROCHE, JEAN, 425

YOUNG, WILLIAM C., see ANTLIFF, HAROLD R., 74

ZARROW, M. X., see PINCUS, GREGORY, 695

SUBJECT INDEX

Adrenal glands
influence on uptake of gold by reticuloendothelial system, 55
inhibitory action of cortical steroids on estrogen-induced uterine growth, 165
regeneration and hypertension as influenced by age, 201
biological activity of derivatives of cortical steroids, 226
effect of unilateral ablation on hydroxycorticoid output, 302
succinate in relation to function of, 317
inhibition of stress-induced ascorbic acid depletion by morphine, *et cetera*, 347
secretion of aldosterone and hydrocortisone after denervation, 360
influence on liver phospholipid metabolism, 364
protection against Brucella somatic antigen in mice after ablation of, 380
function as influenced by ethyl alcohol, 391
influence on gastric pepsinogen in rat, 419
an accessory cortex in monkey kidney, 454
in vitro steroidogenesis as influenced by pituitary trophic hormones, 497
diencephalic regulation of aldosterone secretion, 526
in relation to P metabolism of bone marrow, 656
in vitro steroidogenesis in mouse, 712
Alloxan
effect on guinea pigs with atrophic pancreatic acinous tissue, 131
organ changes first day after injection of, 433
Androgen
induced inguinal hernias in female mice, 48
inhibits anerobic glycolysis of human prostatic adenoma *in vitro*, 159
testosterone inhibits estrogen-induced uterine growth, 165
effect on ovaries and uterus of estrogen-treated hypophysectomized rats, 306
influence on growth and structure of male reproductive accessory glands and on secretion of fructose and citric acid, 479
influence on number of circulating eosinophils in guinea pig, 642
action of 19-nor steroids in female animals, 695.
Antigonadotrophins
effect on splenic ovarian implants, 83

Cortisone
inhibitory action on estrogen-induced uterus growth, 165

Desoxycorticosterone
inhibitory action on estrogen-induced uterine growth, 165

Eosinophils
an explanation of some apparent count anomalies of, 495
as affected in number by testosterone, 642
Epinephrine
Shaw test, 138
interrelation with thyroxine in regulation of O_2 consumption of rat, 217
and related compounds, colorimetric estimation of, 260
Estrogen
effect upon uptake of glycine by connective tissue *in vitro*, 69
influence on sex behavior and tissue responses of male guinea pigs, 74
inhibits anerobic glycolysis of human prostatic adenoma *in vitro*, 159
influence on iodide trapping and protein iodine binding, 289
metabolites in human serum, 499
and aminopterin effects on uterus phosphate metabolism, 505
effect on bone density index, 631
influence on goiter induction, 688

Follicle stimulating hormone
isolation in apparently homogeneous state, 256

Glucagon
effect on glycogen in rats and rabbits, 340
Grafts
of tooth germs in castrate female guinea pigs, 119
Growth hormone
and nutritional status on cardiac glycogen, 241
comparison of two preparations, 356
effect on hematopoiesis in hypophysectomized rats, 444
action on glycosuria and pancreatic islets in partially pancreatectomized rats, 620
content in human pituitaries, 681

Hypophysis, Anterior
influence upon uptake of gold by reticulo-endothelial system, 55
ablation effect on parotid amylase, 254
effect of graded ablation on trophic functions in dog, 293
cytological localization of glycoprotein hormones, 398
inhibition of ablation effects on rat submaxillary glands, 516
reserpine inhibition of stress-induced ACTH secretion, 571
time of release of ovulating hormone from rat hypophyses, 580
P^{32} uptake in brain of normal hypophysectomized and ACTH-treated hypophysectomized rats, 591
and thyroid function in rats with hypothalamic lesions, 593
diabetic action of trophic hormones on adrenalectomized-hypophysectomized dogs, 637
changes in blood ACTH concentration under various experimental conditions, 666
ACTH content in rat, 677
growth hormone content in human being, 681

Luteinizing hormone
assay, as influenced by prolactin, 233

Male sex accessory glands
effect of ablation on "soft calculus" formation in urinary bladder of rat, 257
in vitro observations of intact, castrated and androgen- or estrogen-treated rats, 540

Neurohypophysis
uptake of thyroxine and triiodothyronine, 34
assay of ADS in plasma of normal and diabetes insipidus dogs, 493
and water metabolism in the toad, 601

Pancreas
urinary glucose and tumor growth in diabetic rat, 259
alloxan effect in absence of acinous tissue, 131
hypoglycemic and insulinase inhibitory action of L-tryptophan, 369
structure and function as affected by synthalin A, 708
the hypoglycemic and insulinase-inhibitory action of some plant growth regulators, 715
Parathyroid gland
changes in extracellular calcium phosphorus and citrate ions in nephrectomized rats following ablation of, 27
function and plasma citric acid and calcium response, 181
effect of fluoroacetate upon plasma citrate response to nephrectomy and ablation of, 190
relative effect of vit. D and parathyroid extract on plasma calcium and citric acid of normal and thyroparathyroidectomized dogs, 196
hormone effect on serum glycoprotein and seromucoid levels and on kidney of rat, 532
Prolactin
a factor in LH assay, 233

Relaxin
effect upon uptake of glycine by connective tissue, 69

Sex behavior
as affected by estrogens and problem of hormone specificity, 74
Sex skin
cyclic histochemical changes in, 93
Steroids
21-hydroxypregnane-3, 20-dione sodium succinate biologically inactive, 129
biological activities of derivative of cortical steroids, 226
in vitro effect on electrolyte transfer through frog skin, 458
nephrosclerotic action of fluorocortisol, 646
biological activity of 19-nor compounds in female animals, 695

Testes
grafts induce inguinal hernias in female mice, 48
Thyroid Gland
hormone turnover in cold-exposed rats, 1
altered response and iodine metabolism after I^{131} irradiation, 12
radioiodine not dischargeable with perchlorate in PTU-treated rats, 134
prevention of severe cardiovascular and smooth muscle necrosis by thyro-parathyroidectomy, 273
effect of estrogen on iodide trapping and conversion of inorganic I^{131}
to protein bound I^{131}, 289
iodine metabolism in turtles, 321
effect of estrogen on metabolism of protein-bound iodine, 463
function influence on rat appetite for alcohol, 472
basal metabolism of rats receiving radioactive iodide, 522
alterations of function induced by cobalt, 555
I^{131} metabolism of pre-frozen thyroid slices, 586
function in rats with hypothalamic lesions, 593
xanthine oxidase not present, 596
effect of thiourea on chick embryogenesis, 611
influence of estrogen on goiter induction, 688
Thyroxine
re-evaluation of calorigenic action of L- and D-isomers, 153
effect on latent apyrase activity of mitochondria, 213
interrelation with adrenaline in regulation of O_2 consumption of rat, 217
enzymic conversion to acetic acid analogues, 252
secretion in rats exposed to cold, 323
metabolic actions of derivatives and analogues of, 548
influence on response of kidney tissue metabolism *in vitro*, 719
Triiodothyronine
enzymic conversion to acetic acid analogues, 252
recovery of triiodothyroacetic acid and diiodothyronine from rat kidney after injection of, 425
enhancement of action by phosphate, 565

Uterus
cyclic histochemical changes in guinea pig, 93

Vagina
cyclic histochemical changes in guinea pig, 93

CARCINOMA

Transactions of the 5th Meeting of the International Society of Geographical Pathology

Washington, D.C., 6-11 September, 1954

edited by

Prof. FRED C. ROULET (Basel)

Secretary-General of the Society

572 p., 182 figs., U.S. $16.20

The Transactions publish in extenso all reports and individual contributions (about 60) presented at the Congress in the original language.

Main Subjects

Geographical Pathology of Cancer in General—Carcinoma of the Liver—Carcinoma of the Stomach—Carcinoma of the Uterus—Carcinoma of the Breast—Cancer of the Lung

---

Gold Treatment of Rheumatoid Arthritis

with some Notes on Hormone-Gold and Hormone-Salazopyrin Therapy

edited by

Folke Bohman (Uppsala)

from the Royal Pensions Board's Hospital, Nynäshamn, Sweden (Head: Frederik Sundelin, M.D.), and the State Institute of Human Genetics and Race Biology, Uppsala

165 p., 62 tab. U.S. $4.15

Review of the Literature, **Introduction, Gold Therapy, Physical Therapy, Other Modes of Treatment, Cause of Death in Rheumatoid Arthritis—***Materials for Study—Immediate Results of Therapy—Patients Given More than One Course of Treatment—Mortality—End Results—Age and Therapy—Gold Treatment—Working Capacity—Appendix—* **Review of the Literature on Hormones Alone and Combined with Other Remedies—Hormones Combined with Gold in the Treatment of Rheumatoid Arthritis—Combined Hormone-Salazopyrin Treatment—***The Results Obtained—Bibliography*

BASEL 11 (Switzerland) S. KARGER NEW YORK

For U.S.A.: Albert J. Phiebig, P.O. Box 352, White Plains, N.Y.

THE ENDOCRINE SOCIETY

LAWSON WILKINS, *President* — ELEANOR VENNING, *Vice-President*
LEO T. SAMUELS, *President-Elect* — HENRY H. TURNER, *Secretary-Treasurer*

COUNCIL: ROY HERTZ, JOSEPH W. JAILER, ELEANOR VENNING, EARL T. ENGLE, LEONARD I. PUGSLEY, ALLAN T. KENYON, WARREN O. NELSON, E. B. ASTWOOD, HENRY H. TURNER, LEO T. SAMUELS, A. ALBERT, LAWSON WILKINS.

PUBLICATION COMMITTEE: F. D. W. LUKENS (*chairman*), E. B. ASTWOOD, RULON RAWSON, E. P. MCCULLAGH, ROY HOSKINS, JOHN EAGER HOWARD, H. H. TURNER.

ENDOCRINOLOGY

Editorial Board

A. ALBERT
F. C. BARTTER
R. GAUNT
R. HERTZ
W. R. LYONS
EVELYN ANDERSON
J. T. BRADBURY
N. S. HALMI
D. J. INGLE
L. I. PUGSLEY
A. E. WILHELMI
B. L. BAKER
R. I. DORFMAN
R. D. H. HEARD
C. P. LEBLOND
C. H. SAWYER

ROY O. GREEP, *Managing Editor*
25 Shattuck St., Boston 15, Massachusetts

ENDOCRINOLOGY is published monthly by The Endocrine Society. Annual subscription price is as follows: In the United States, U.S. Possessions, Pan-American Union & Spain, $15.00; Canada, $15.50; other foreign countries, $16.00. Single copies, $1.50 postpaid. Distributed in the British Commonwealth of Nations by Blackwell Scientific Publications, Ltd., 24-25 Broad Street, Oxford, England. Subscription price £5.15s.0d. Copyrighted in the British Commonwealth by Blackwell Scientific Publications, Oxford. No part may be duplicated or reproduced without permission of publishers.

INSTRUCTIONS TO AUTHORS

Preference will be given to papers which are contributive to fundamental knowledge of the endocrines. The JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, also published by The Endocrine Society, is held to be a more suitable outlet for papers on clinical endocrine medicine and case reports. Papers should be written with the greatest concision compatible with clarity and accuracy. Papers presenting negative or confirmatory results are considered only for the section on NOTES and COMMENTS. Submission of a paper to The Endocrine Society's journals is held to imply that it is not to be published elsewhere. Papers are published in the order of their acceptance with the exception that those given a low priority rating by the Editors may be delayed at the discretion of the Managing Editor.

NOTES and COMMENTS, a section appearing at the end of most issues is designed primarily for the prompt publication of material of unusual interest. Other papers suitable for this section include those in the nature of progress reports, those describing new methods, techniques or apparatus, and those describing results of a confirmatory or negative nature. Manuscripts must not exceed four double-spaced typewritten sheets. Tabular and illustrative material must be kept to a minimum.

NEW BOOKS will be listed as they are received and those which bear on topics related to endocrinology will be reviewed as promptly as possible and as space permits.

Reprints: A price list and order blanks for reprints will be sent with the Galley proofs.

Preparation of Manuscripts: *Specifications:*

1. Double-spaced or triple-spaced typewritten copy should be submitted in duplicate.
2. Tables, references, legends and footnotes should be on separate sheets.
3. In the text indicate citations to literature by number (in parentheses) and list references in numerical order at the end of the paper. Abbreviations of the names of journals should correspond to the system used by the Quarterly Cumulative Index Medicus. References should be typed as follows: Taurog, A., I. L. Chaikoff and D. D. Feller: *J. Biol. Chem.* 171: 189. 1947. The references cited should have an important bearing on the work being reported.
4. The following type of abbreviation should be employed: gm., mg., μg., cc., m., cm., mm., μ, μμ, C., m.eq., R.U., U., I.U.
5. If the proprietary names of substances employed are to be given they should be appropriately identified as such.
6. Use "Fig." in parentheses and at the beginning of a legend; as a part of the text use "Figure," "Table" and "Plate."
7. A summary in briefest possible form should close the article.
8. Provide a running page title (not over 35 letters and spaces).

Photographs, preferably supplied unmounted, must be trimmed and arranged to conserve vertical space. Graphs or line drawings should be on white board or blue-line paper. All lettering must be done in black ink and be suitably proportioned to print legibly. Photographs of graphs and drawings that are of inconvenient size for mailing will ordinarily be suitable. Colored photographs are published at the author's expense.

For each manuscript, authors will be allowed the equivalent of $25.00 for the reproduction of illustrative material and will be allowed one page of tabular material without charge. Important additional matter of this sort may be allowed at cost at the discretion of the Managing Editor. The cost of making excessive alterations in Galley proof will be charged to the author.

CORRESPONDENCE

Manuscripts, correspondence relating thereto and new books for review should be addressed to ENDOCRINOLOGY, Editorial Office, *25 Shattuck Street, Boston 15, Massachusetts.*

Correspondence relating to membership applications and Society business should be sent to Dr. Henry H. Turner, *1200 North Walker St., Oklahoma City, Oklahoma.*

All other business matters, including correspondence and remittances relating to subscriptions, back volumes and advertising, orders and remittances for reprints and membership dues should be sent to CHARLES C THOMAS, PUBLISHER, *301-327 East Lawrence Avenue, Springfield, Illinois.*

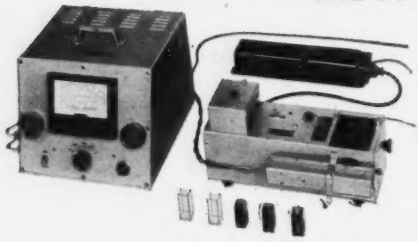
Entered as second-class matter at the Post Office at Springfield, Illinois, and at Menasha, Wisconsin, under the act of March 3, 1879. Accepted for mailing at special rate of postage provided for in the Act of February 28, 1925 embodied in Paragraph 4, Section 538, P.L. & R.

Copyright 1956, by The Endocrine Society. Printed in U.S.A.

PHOTOVOLT

Line-Operated Multiplier FLUORESCENCE METER

Mod. 540



- **High-sensitivity for measurement of low concentrations (full-scale setting for 0.001 microgram quinine sulphate)**
- **Micro-fluorimetry with liquid volumes as low as 1 ml**
- **Low blank readings, strict linearity of instrument response**
- **Interference filters for high specificity of results and for determining spectral distribution of the fluorescent light.**

Write for Bulletin No. 392 to

PHOTOVOLT CORP.

95 Madison Ave. New York 16, N.Y.

An Authorized Binding for
ENDOCRINOLOGY

Arrangements have been made for subscribers to have their journals bound into distinctively designed books.

Six issues, January through June or July through December, bound in best grade navy washable buckram, imprinted with your name on cover, cost but $3.75 per volume.

Bound journals serve as an immediate reference for research and information. Properly displayed, they create a psychological impact on the patient, implying the time and effort spent to keep up-to-date on the most modern techniques and treatment.

Ship journals parcel post. Within thirty days after receipt, bound volumes will be shipped prepaid anywhere in the U.S.A. Full remittance must accompany order.

PUBLISHERS' AUTHORIZED BINDERY SERVICE
(Binders of all Journals)
5811 W. Division Street
Chicago 51, Illinois

Mail this form to Charles C Thomas · Publisher · Springfield · Illinois and you will receive your copy immediately.

TRANSACTIONS OF THE AMERICAN GOITER ASSOCIATION—1955

•

Many communications have been received indicating that this meeting was an outstanding one, particularly because of the excellence of the papers, dealing with biochemistry and its allied sciences. An internist interprets the results of the various laboratory procedures in terms designating in what manner they might be of practical value.

Complete the form below and we will send you one copy of Transactions of the American Goiter Association ($14.00, 482 pages, 196 illustrations), for ten days free inspection approval.

•

Name .....
Address .....
City .....Zone .....
State .....

A few copies of the 1948, 1949, 1950, 1951, 1952 and 1953 Transactions are still available on the same terms.

•

Check the issues you want sent:

- ☐ **1948**—320 pp., 100 illus., $6.50
- ☐ **1949**—476 pp., 160 illus., $10.50
- ☐ **1950**—455 pp., 198 illus., $11.50
- ☐ **1951**—525 pp., 222 illus., $12.50
- ☐ **1952**—446 pp., 159 illus., $12.50
- ☐ **1953**—440 pp., 145 illus., $13.50
- ☐ **1954**—496 pp., 52 illus., $14.00
- ☐ **1955**—482 pp., 196 illus., $14.00

Real Values For Endocrinologists

1. **HYPERPARATHYROIDISM.** By B. Marden Black, Mayo Clinic. Pub. '53, 127 pp., 24 il., (Amer. Lec. Endocrinology), Lexide, $3.75

2. **STUDIES ON TESTIS AND OVARY, EGGS AND SPERM.** Edited by Earl T. Engle. Pub. '52, 256 pp., 96 il., Cloth, $7.75

3. **LABORATORY AIDS IN ENDOCRINE DIAGNOSIS.** By Roberto F. Escamilla, Univ. Calif. Pub. '54, 152 pp., 16 il., (Amer. Lec. Endocrinology), Cloth, $4.75

4. **SIMMONDS' DISEASE: Extreme Insufficiency of the Adenohypophysis.** By R. F. Farquharson, Univ. Toronto. Pub. '50, 104 pp., 5 il., (Amer. Lec. Endocrinology), Lexide, $2.00

5. **HYPOTHALAMIC - HYPOPHYSIAL INTERRELATIONSHIPS: A Symposium.** Third Annual Scientific Meeting of the Houston Neurological Society, Texas Medical Center, Houston, Texas. Chairman, Hebbel E. Hoff. Compiled and edited by William S. Fields, Roger Guillemin, Charles A. Carton, all of Baylor Univ. Pub. '56, 176 pp., 79 il., Cloth, $4.75

6. **COMPARATIVE PHYSIOLOGY OF THE THYROID AND PARATHYROID GLANDS.** By Walter Fleischmann, Johns Hopkins Univ. Pub. '51, 85 pp., (Amer. Lec. Endocrinology), $2.50

7. **HORMONES AND BODY WATER.** By Robert Gaunt and James H. Birnie, Syracuse Univ. Pub. '51, 68 pp., 5 il., (Amer. Lec. Physiology), Lexide, $2.25

8. **HYPOGLYCEMIA AND THE HYPLOGLYCEMIC SYNDROME.** By A. J. Kauvar, Univ. Colo., and Martin G. Goldner, State Univ. N.Y. Pub. '54, 88 pp., 12 il., (Amer. Lec. Endocrinology), Lexide, $3.00

9. **SUBACUTE BACTERIAL ENDOCARDITIS.** By Andrew Kerr, Jr., Louisiana State Univ. Pub. '56, 368 pp., 16 il., (Amer. Lec. Internal Medicine), Cloth, $6.50

10. **THYROID FUNCTION AND ITS POSSIBLE ROLE IN VASCULAR DEGENERATION.** By William B. Kountz, Washington Univ. Pub. '51, 71 pp., 10 il., (Amer. Lec. Circulation), Lexide, $2.25

11. **SURGERY OF THE ADRENAL GLANDS.** By William Wallace Scott, Johns Hopkins Univ. Pub. '54, 136 pp., 60 il., (Amer. Lec. Abdominal Viscera), Lexide, $3.50

12. **TREATMENT OF TOXIC GOITER WITH RADIOACTIVE IODINE.** By Lindon Seed, Univ. Illinois; and Theodore Fields, Northwestern Univ. Pub. '53, 128 pp., 17 il., (Amer. Lec. Endocrinology), Lexide, $3.75

13. **HYPOTHYROIDISM: An Essay on Modern Medicine.** By Paul Starr, Univ. So. Calif. Pub. '54, 144 pp., 25 il., (Amer. Lec. Endocrinology), Lexide, $3.75

14. **STUDIES ON FERTILITY: The Proceedings of the Society for the Study of Fertility:** Volume VI (1954). Edited by R. G. Harrison. Pub. '55, 162 pp., 90 il., Cloth, $4.25

15. **STUDIES ON FERTILITY: The Proceedings of the Society for the Study of Fertility:** Volume VII (1955). Edited by R. G. Harrison. Pub. '56, 166 pp., 202 il., Cloth, $5.00

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

CHARLES C THOMAS • PUBLISHER

Springfield • Illinois

Send me the books for which I have checked the identification numbers on 10 days free inspection approval. I will send you a remittance within 30 days for the books I decide to keep. (1005)

Check here for BOOKS *you want*

Name_____

Address_____

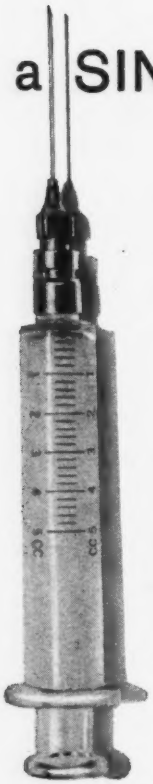
City_____ Zone_____ State_____

treat adrenocortical insufficiency

without the
difficulties
of
implants

without the
unpleasantness
of daily
injections

with a SINGLE injection a month



Percorten®

trimethylacetate

(desoxycorticosterone trimethylacetate CIBA)

Percorten trimethylacetate, developed after more than 3 years of research by CIBA, provides expedient, lasting hormonal support for the patient with adrenocortical hypofunction . . . avoiding both the uncertainty of surgical implantation and the vexation of daily injections. With once-a-month therapy, a single injection will produce prolonged activity without acute signs of overdosage.[1]

Multiple-dose Vials, 4 ml., containing 25 mg. Percorten trimethylacetate per ml. as an aqueous microcrystalline suspension for intramuscular use only.

1. Frawley, T. F., and Forsham, P. H.: J. Clin. Endocrinol. *11*:772 (July) 1951.

CIBA
SUMMIT, N. J.

5 485

y

n